AIDS TO BACTERIOLOGY

MOOR & PARTRIDGE

THIRD EDITION

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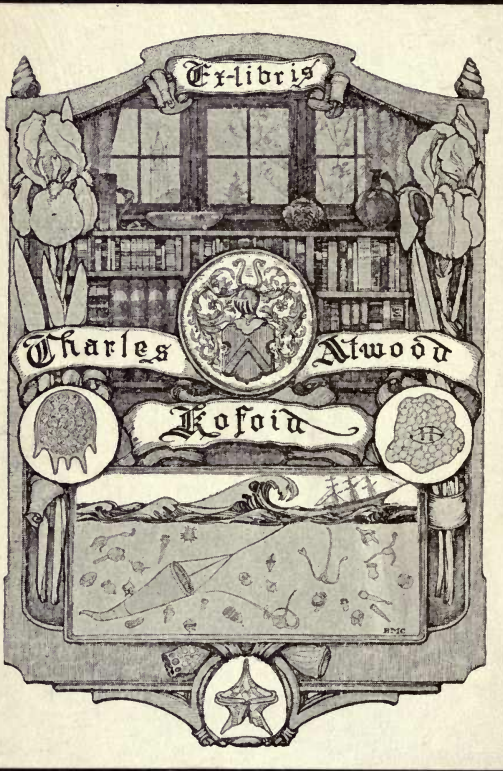
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TO
BACTERIOLOGY

BY

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THIRD 

EDITION

LONDON
BAILLIÈRE, TINDALL & COX
8, HENRIETTA STREET, COVENT GARDEN
1916

PRINTED IN GREAT BRITAIN.

PREFACE TO THE THIRD EDITION

While no notable discovery has been recorded in bacteriology since the appearance of the second edition of this book, much extension and consolidation of previous knowledge have been effected. This has necessitated revision and enlargement of nearly all parts of the book.

A war on highly-cultivated soil, with troops occupying the same ground for lengthy periods, has very forcibly obtruded the pathogenic abilities of many fæcal bacteria. Tetanus, typhoid, paratyphoid, and dysentery bacilli, the bacillus of malignant œdema, and the fæcal pyogenic streptococci, have all been in evidence. *Bacillus Welchii*, under one or other of its many names, has also claimed much attention in connection with gaseous gangrene. In the case of these organisms, however, new developments have been chiefly in the directions of pathology and treatment rather than in bacteriology.

We have considerably extended our article on and devoted an appendix to the meningococcus—an organism whose life outside the human body remains largely a matter for conjecture. We have collected in one chapter a summary of the little that is known about the filterable viruses, and have also dealt with anaphylaxis and the preparation of vaccines. In accordance with the advice of Professor David Ellis, we have substituted the classification of Migula for that of Hueppe.

Few bacteriological investigations are free from possible fallacy or insusceptible of wrong deduction. The influence of anti-typhoid inoculation on the Widal test, the absence of tubercle bacilli in milk from tuberculous udders, the absence of colon bacilli from polluted water, the incorrect selection of organisms in the preparation of bacterial vaccines, and the fact that the popular Wassermann

reaction is not a true antigen test, are but a few instances of many which occasionally intrude to mislead the unwary bacteriologist and to confuse those who rely on his findings. So far as space allows, we have briefly indicated where, why, and how, errors may be expected and avoided.

Surgeons on military service, when first called on to deal with the complicated wounds caused by the pointed-nose bullet and contaminated with resistant fæcal organisms carried in from soil and clothing, found methods then current for antisepsis quite inadequate. Discussion of the relative values of antiseptics for surgical use continues, the tendency at present being to use those dependent for action on the oxidising properties of chlorine. Sir Almroth Wright tackles the problem of cleansing wounds by the application of very strong saline solution, which results in a considerable exudation of serum. Though it has no germicidal action for the streptococci, this process is much used, invoking both praise and criticism. Magnesium sulphate and glycerin are also used, and, it is said, kill the streptococci (A. E. Morison's process).

Bacteriology promises to modify agricultural methods, and in Chapter XX. we give résumés of what has been accomplished in soil-sterilisation and nitrogen-fixation.

While most bacteriologists have had some personal experience of bacterial mutability, to which we make frequent reference, this remains largely of academic interest. In practice, species tend to crop up fairly true to type, and exceptional conditions are usually required to produce important change in attributes.

C. G. M.
W. P.

London,
May, 1916.

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CHAPTER I

INTRODUCTION

THE Thallophyta, which form the lowest group of cryptogamic plants, show no division into root and stem, and have no fibro-vascular system. They are divided into algæ, which contain chlorophyll, and fungi, which contain none. Excluding the Hymenomycetes (mushrooms, etc.), the fungi are microscopic, and most genera and species come within the purview of bacteriology. The Hyphomycetes (moulds) and the Blastomycetes (yeasts and torula) receive frequent attention from the bacteriologist, and Chapters XV. and XVI. are devoted to them. While the study of bacteria, or Schizomycetes, is the primary object of bacteriology, its scope has gradually extended beyond the fungi to include organisms too small to be seen with the microscope (ultra-microscopic organisms) and such unicellular animals (Protozoa) as cause disease in man, beast, or plant. Of vital importance is a knowledge of the circumstances under which organisms die, so disinfection occupies a prominent place in the science.

Anatomy.—The cell wall is a true membrane (Ellis and Meyer), and is generally composed of a nitrogenous substance very similar to chitin, a skeletin not found in vertebrates, but of frequent occurrence in the cuticles of invertebrates.

The cell membrane in many, perhaps all, forms sometimes swells to form a *capsule*. Large numbers of bacteria may cluster together in a jelly-like mass known as a *zooglœa*.

The cell contents, or *cytoplasm*, consists of translucent protoplasm. Scattered through the cell or massed at the poles of certain bacteria are granules that stain differently

to the remainder of the cell (metachromatic granules). These are composed of glycogen, fat, or, according to Dobell, nucleic acid combined with an organic base. They are probably in large part reserve food substance (Jordan).

The presence of a nucleus in all species is regarded as proved by the work of Clifford Dobell.

Movement.—Many bacteria, particularly bacilli and spirilla, are capable of motion, produced by the little protoplasmic threads (flagella). Ellis has shown that flagella arise from the cell substance and not from the membrane.

Some organisms, such as the cholera spirillum, have a single flagellum at one end (*monotricha*), in others there is one at each pole (*amphitricha*); the flagella may assume the form of a tuft at one pole (*lophotricha*), or they may be scattered round the cell (*peritricha*). The position of the flagella is always the same in each species; but in those species having more than one flagellum the number is not always constant, and depends on the health of the culture (Ellis). Even these organisms are not always motile, but go through a resting stage. Motility is best seen in young cultures when conditions are favourable for growth. Flagella are not seen when the organism is examined under the microscope in the usual way, but they can be observed in specimens specially stained. This independent movement must not be confused with the motion that solid particles, whether bacteria or not, exhibit when suspended in a fluid medium, known as the ' Brownian movement,' which is variously attributed to electrical disturbances and to surface tension.

Size.—The unit of measurement adopted is the ' micron ' (often erroneously called a micro-millimetre), which is equal to 0·001 millimetre, and is represented by the letter μ. The influenza bacillus measures about $0{\cdot}5 \times 0{\cdot}2\ \mu$, while the spirochæte of relapsing fever may attain $40\ \mu$ in length. Great differences in length are not observed among the majority of species, most of the bacilli, for instance, measuring about $2\ \mu$. Even smaller than the influenza bacillus is the organism causing pleuro-pneumonia in cattle, which is just visible under the highest powers. ' Ultra-microscopic ' organisms capable of passing through the filter mass of a porcelain filter cause many diseases (see Chapter XIX.).

Reproduction.—Bacteria are asexual, and propagate by fission. When a cell has attained the maximum size for its species, it elongates, with constriction round its middle, followed by a simple partition. Hence the family name of Schizomycetes. Two young cells are thus formed from the mother cell. This process may be repeated as often as once in twenty minutes if conditions are favourable. An increase in geometric progression is not consistently maintained, however, owing to various checks on the growth. While insufficient food, lack of moisture, and other conditions, disallow unhampered multiplication, the chief hindrance is the production of inhibitory substances by the vital activity of the bacteria themselves.

When bacteria have occupied the same site in the body or lived in the same culture for a long period, or conditions for growth are otherwise unfavourable, abnormal shapes and sizes are produced (involution forms). These generally take stain less readily, or else do not stain at all. Other characters, such as pathogenicity or fermentation power, are likely to diminish, and there is general evidence of degeneracy.

The cocci do not always separate after fission; division may occur in one plane with the formation of a chain (*streptococci*); in two planes, producing a cluster (*staphylococci*); or in three planes, forming cubical bales (*sarcina*).

Spore-formation is not common; it occurs most frequently in bacilli and spirilla, and rarely in micrococci.

Endogenous Spores.—The protoplasm becomes granular, and some of the granules coalesce to form a highly refractile round or ovoid body enclosed in a tough membrane. Spores exhibit very great resistance to heat, desiccation, and chemical agents. They are thus able to preserve their species through most disadvantageous circumstances. When favourable conditions recur, the spore loses its refractile appearance, elongates, and bursts its membrane to extrude an organism which divides in the usual manner. Only one spore is formed in a cell, so that this cannot be regarded as a reproductive process. While in most cases the diameter of the spore does not exceed that of the parent organism, it may be greater, and will give a 'drumstick' appearance if terminal and round; or it may be swollen and club-shaped (*clostridium*). The conditions determining the formation of spores are variable for different

organisms. The anthrax bacillus spores when in contact with free oxygen, while the anaërobes generally require absence of oxygen. An organism containing no spore, but ready to divide by fission at maturity, is said to be a *vegetative form.*

Arthrospores.—Some of the cells formed by fission were formerly thought to possess the characters of spores. The formation was known as 'arthrogenous,' and has only been noticed in the micrococci. The suggestion is based on a misconception. Eyre states that these so-called arthrospores have never been observed to germinate, and they cannot survive a temperature of 80° C. for ten minutes. It is now universally accepted that these individuals are not spores.

The formation of spores in imperfectly divided sister cells has been interpreted, wrongly, according to most authorities, as 'autogamy.' There is no evidence that either this or other sexual process occurs among bacteria.

Bacteria cannot arise *ab initio*, since a living organism can only be derived from a living organism (biogenesis). The supporters of the theory of 'spontaneous generation' (abiogenesis or archebiosis) believed that organisms could be produced in a sterile medium without the introduction of living organisms.

Classification.—The bacteria possess so few morphological attributes, and so many forms are pleomorphic, that the ingenuity of bacteriologists has hitherto been incapable of formulating either a scientific or a convenient classification. The present nomenclature is more of a hindrance than a help, for so many organisms are crowded together in a single genus, many of them possessing names either unwieldy or unsuitable, that but little assistance can be expected from the classification. We append one scheme, that of Migula, and comment on it where necessary:

The bacteria are divided into five families: Coccaceæ, Bacteriaceæ, Spirillaceæ, Chlamydo - Bacteriaceæ, and Beggiatoaceæ. These again are subdivided into genera, based partly on the mode of division and partly on the number and arrangement of the flagella.

I. Coccaceæ.—Round or oval cells. The division into genera is based on the arrangement of the cocci on division.

(1) *Streptococcus.*—The cocci form chains. Includes cocci forming pairs (Diplococci).

(2) *Micrococcus.*—Irregular grouping. Micrococci forming clusters in an arrangement suggestive of that of grapes on a bunch are still known as Staphylococci, though there is a movement among the precise to eradicate the word.

(3) *Sarcina, or Packet Cocci.*—Division in three directions, forming packets of eight or more elements, which remain associated in more or less cubical masses. Non-motile.

(4) *Planococcus* } Motile forms.
(5) *Planosarcina* }

II. BACTERIACEÆ.—Straight rod forms.

(1) *Bacterium.*—Non-motile organisms. The name has been variously applied in other systems of classification. In one it signified a very short rod, and in another a rod of a non-sporing species. In its plural form it is used as a general term for all the organisms included in this table; otherwise it has fallen into disuse.

(2) *Bacillus.*—Migula applied the term to rods possessing both polar and lateral flagella. It is now customary to apply the name to all rod-shaped organisms—*i.e.*, all of the *Bacteriaceæ.*

(3) *Pseudomonas.*—Rods with polar flagella only. The title is now seldom used except for some bacilli concerned in the nitrogen cycle, and for others concerned in plant diseases.

III. SPIRILLACEÆ.—Curved or spiral rod forms.

Migula divides this family into three genera: non-motile organisms (Spirosoma); motile forms with a single polar flagellum (Microspira); and motile forms with more than one polar flagellum (Spirillum). Other classifications apply the name Spirillum to a larger class, causing much confusion in nomenclature. The result is that any member of the family may now be properly referred to as a spirillum, though the term is often restricted to those organisms having three or more definite corkscrew turns. Short curved rods are commonly termed ' vibrios,' or ' comma ' bacilli, but the latter name is frequently used for, and should be restricted to, the cholera vibrio.

IV. CHLAMYDO-BACTERIACEÆ.—Thread forms not containing sulphur granules.

(1) *Streptothrix.*—Forms showing true but not dichotomous branching.

(2) *Crenothrix.*—Threads thicker at apex than at base. Each thread is tubular, and inside each a linear series of cells is arranged, each cell possessing a membrane of its own. The cells are thrown out at the top, and elongate to form new threads like those of the parent plant.

(3) *Leptothrix.*—Threads with or without sheaths, showing no branching.

(4) *Cladothrix.*—The threads possess pseudo-branches. Strings of rod-like cells are enclosed in a sheath.

V. BEGGIATOACEÆ.—Thread forms containing sulphur granules.

(1) *Beggiatoa.*—Long motile free-swimming threads of colourless cells containing strongly refracting granules of sulphur.

(2) *Thiothrix.*—Threads that differ from beggiatoa by having the filaments attached at one end.

Organisms other than the true bacteria are dealt with later, and descriptions of them are left to the special monographs and chapters.

Growth of the Bacteria.—Few bacteria can derive their nourishment from inorganic sources, and an absence of chlorophyll prevents all species from utilising atmospheric carbon dioxide. The large majority require complex organic compounds such as proteins and carbohydrates as well as mineral salts for food. Suitable mixtures are prepared for laboratory purposes (culture media—*v.* Chapter III.). While some simple media allow growth of most species, by the introduction of other constituents certain bacteria are favoured and flourish, perhaps with specific indications of their presence, while other species are killed or suppressed (selective media). By studies of the preferences and conditions of vigorous growth in this and other respects, the extraction of a required organism from admixture with others is facilitated.

Bacteria derive their oxygen either from the air (aërobes) or from compounds containing oxygen (anaërobes). The 'facultative anaërobes' grow either in the presence or absence of oxygen. There are gradations in this respect, from the strictly aërobic species, which require abundance of oxygen, and will not grow in its absence, to the anaërobic, which grow in the absence of free oxygen. Strict anaërobes do not exist. Organisms that can live without oxygen thrive better when oxygen is present, but in very small quantity.

Moore and Stenhouse Williams killed tubercle bacilli by three weeks' exposure to an atmosphere enriched with oxygen, and found the plague bacillus and *staphylococcus* growths were adversely affected (oxyphobia).

As a rule, bacterial growth ceases at temperatures below 12° C. and above 42° C. The range of temperature within which bacteria will grow is practically constant for each species, but there is a more narrow margin (the 'optimum' temperature) in which each does best. The optimum temperature differs more or less according to the species. Normal inhabitants of the human body and organisms pathogenic for man thrive best at blood-heat (37° C.), the colon bacillus, which grows luxuriantly at 42° C., being an exception. Bacteria obtained from the lower animals generally have the normal temperature of the host as an optimum. The body-temperature of a fowl is 42° C., and avian tubercle bacilli thrive at 43° C., a temperature at which human tubercle bacilli refuse to grow.

Some bacteria isolated from dung and from heated hay grow best at temperatures between 60° and 70° C. (thermophilic organisms), while others can grow at 0° C. Growth at unnatural temperature may result in loss of some characters.

When obtaining their nourishment from some living body or 'host,' organisms are known as 'parasites.' The adjective 'obligate' is prefixed if they can live only on this 'host.' If the bacteria grow on dead organic matter, they are called 'saprophytes.' These are also divided into 'obligate' and 'facultative' saprophytes. The term 'obligate parasite' requires to be used with some reservation. It merely indicates that hitherto attempts to cultivate an organism on culture media have failed, and a successful attempt thereat automatically transfers it to the list of facultative parasites.

Resistance to External Influences—*Cold.*—The germicidal effect of low temperatures is small. Growth usually ceases below 10° C., but even after exposure to − 252° C. (the temperature of liquid hydrogen) for ten hours, or to −170° C. for several weeks, bacteria are not killed, and when placed in favourable conditions show that no serious impairment has taken place. When water freezes naturally there is a high death-rate among the bacteria therein, and repeated freezing and thawing has been found more

destructive to the typhoid bacillus than continuous freezing. S. C. Keith states that living bacteria are hardly ever found in clear ice, though they are comparatively abundant in snow ice and bubbly ice. Cooling is valuable in the preservation of putrescible material, because it inhibits bacterial growth.

Desiccation.—Moisture is absolutely necessary for the growth of bacteria. Ordinary drying in the air has a detrimental effect on the vegetative forms of bacteria, spores suffering less or not at all. Resistance varies with the species. The tubercle bacillus, which retains its virulence after five months' desiccation, is much more resistant than the cholera spirillum, which is incapable of development after three hours' drying in the form of a very thin film. In spite of the large numbers of colon bacilli continually being deposited in our streets, Gordon failed to find the organism in 500 litres of air of the East Central district of London.

Heat.—The *Thermal Death-point* (Eyre) for vegetative forms is 'that temperature which with certainty kills a watery suspension of the organisms in question after an exposure of ten minutes.' Eyre defines the moist *t.d.p.* for spores as 'that time exposure to a fixed temperature of 100° C. necessary to effect the death of all the spores present in a suspension.' Eyre's dry thermal death-point for both vegetative forms and spores is the temperature that kills each form in a thin film after a time exposure of ten minutes. The thermal death-point can be determined with a fair degree of accuracy for each species, and is much higher for spores than for the vegetative forms. Heat may be applied either in the absence or presence of moisture, known respectively as 'dry heat' and 'moist heat.'

Dry heat is much less efficient than moist heat, the death of the protoplasm taking place more readily in the presence of moisture. In practical disinfection moist heat is therefore used where possible, especially where penetration of fabrics is required (see p. 245). Steam may be applied under pressure, as in an autoclave, when a fifteen minutes' exposure to 125° C. suffices to destroy all known organisms. When moisture is present, most vegetative forms are killed by an exposure to 65° C. for ten minutes, while an exposure for an hour and a half at

120° C. to 128° C. is necessary to attain the same result with dry heat. While the spores of most pathogenic bacteria are destroyed by boiling for a short time, those of non-pathogenic species require a much longer exposure. A temperature of 140° for three hours is necessary to secure destruction of the spores of some organisms by dry heat. *B. subtilis* spores are particularly resistant, and Ellis found they resisted boiling at 100° C. for six hours without injury to their germinating power.

Fractional Sterilisation.—Instead of performing sterilisation in one operation, 'discontinuous' or 'fractional' sterilisation may be employed. For this the medium or other material is exposed to heat for a few minutes on two or three successive days. Any spores surviving the first heating germinate, and the resulting organisms succumb to the second and third sterilisations. Hewlett ascribes some of the sterilising effect simply to the injurious action of alternate heating and cooling. As spores may take days or even weeks to germinate, the procedure is not always certain in its action, and it is thus possible for spores to germinate in a medium believed to be sterile.

Light.—Sunlight and, to a less degree, the electric arc are very injurious to certain forms of bacteria. The red and yellow rays of the spectrum have little effect, germicidal action being exerted by the blue and violet portions. The violet rays are therapeutically applied in the Finsen light treatment of lupus vulgaris. Water and the ordinary milk of commerce can be sterilised without appreciable rise of temperature by exposure to the ultra-violet rays of a quartz-mercury lamp. Hewlett and Barnard find that these rays have practically no power of penetration, and are stopped even by thin glass. The action of light is superficial, even a short depth of water stopping the action. Probably sunlight does not materially assist the purification of rivers. Definite germicidal action cannot yet be attributed to the Röntgen rays, while radium emanations require long exposure and close contact to exert appreciable action.

Pressure.—If suddenly applied or released, pressure may rupture bacterial cells; otherwise no appreciable effect has been observed.

Electricity.—The products of electrolysis may destroy bacteria, and currents of high potential may inhibit

growth. C. Russ (*Proc. Roy. Soc.*, 1909, p. 314) shows that under the influence of a suitable current certain bacteria aggregate at one or other electrode, and uses this action for the collection of bacteria from a fluid medium. Thus, if sodium chloride be used, nearly all go to the positive electrode, a large number being killed. It is suggested that successful ionisation of suppurating wounds is effected by drawing the bacteria out of the tissues and then exposing them directly to the destructive effect of the electric current.

Products of Metabolism in Bacteria.—The action of bacteria on the surrounding medium is generally of an analytical nature, complex nitrogenous substances and carbohydrates being decomposed into simpler substances. Jordan attempts to classify the chemical products of bacteria under the following heads: (i.) The secretions or substances which subserve some purposeful end in the cell economy; (ii.) the excretions or substances ejected because useless; (iii.) the disintegration products formed by the breaking down of food substances; and (iv.) the true cell substance. The word 'aporrhegma' has been applied to any substance split off by biological actions, but its employment is not encouraged by the precise.

Production of Heat.—Bacteria of thermophilic character are responsible for self-heating of hay and some forms of spontaneous combustion, especially the firing of moist cotton.

Photogenesis.—Phosphorescence, especially that seen on fish, is often due to bacteria. The photogenic bacteria are generally, but not always, of marine origin.

Some bacteria produce a fluorescence in the culture medium.

Chromogenesis.—A number of bacteria produce pigments, often lipochromes. The *raison d'être* of these pigments is uncertain, but probably they are excretory products of no service to the organism. *B. prodigiosus* produces a red pigment. On one occasion it contaminated a water-supply and infected the bread made with it. The same organism is the causative agent of the 'bleeding host.' Chromidrosis (coloured sweat) is attributed to bacteria. While the term 'chromogen' is restricted to bacteria that produce a pigment either for retention in their cells or for excretion, many others produce a pigment or change of colour in

culture media. Beyerinck gives some instances that result from oxidation by bacterial action: The oxidation of quinic acid to protocatechuic acid is brought about by *Micrococcus calco-aceticus* and *B. fluorescens non-liquefaciens*. Quercitol is oxidised to pyrogallol by *Pseudomonas aromatica*. Melanine can be formed from tyrosine by *Vibrio tyrosinatica*, isolated from sea-water and sewage. *Acetobacter melanogenum*, occurring in vinegar, converts peptone into a caramel-like substance.

Fermentation.—By bacteria (Chapter XVIII.); by moulds (Chapter XVI.); by yeasts (Chapter XV.); and by enzymes, or 'zymolysis' (Chapter XVIII.).

Putrefaction.—Nitrogenous substances, such as the proteins, are decomposed by bacteria, particularly by those of the *Proteus* group. The insoluble albumins, etc., are first converted into albumoses and peptones; then amino-acids are produced, and a variety of other substances, such as fatty acids, basic bodies, and gases.

Indole.—Indole is one of the final products of the decomposition of proteins, and is of importance in bacterial diagnosis, as organisms, otherwise very similar, may differ in regard to the production of this substance. To ascertain if an organism produces indole, it is inoculated into peptone water (2 per cent.) or into a glucose-free broth. The culture is incubated for twenty-four hours or longer. Two c.c. of a stock solution of sodium nitrite (5 per cent.) are diluted to 100 c.c. One c.c. of this weak solution is added to the culture (the volume of which should be about 10 c.c.). A little concentrated hydrochloric acid is then allowed to trickle down the wall of the inclined test-tube to liberate nitrous acid, which gives a pink colour with the indole. If a definite reaction is not obtained, the tube is placed in the blood-heat incubator for half an hour to intensify the colour. At the same time as the peptone water is inoculated, another tube should be infected with an organism known to produce indole, and an uninoculated control-tube placed with the others in the incubator. The purity of the reagents and the power of the peptone to allow production of indole is thus secured. As a more delicate reaction for indole the following reagent may be used: 4 grammes of paradimethylamidobenzaldehyde are dissolved in 380 c.c. of absolute alcohol and 80 c.c. of concentrated hydrochloric acid. To 2 c.c. of the culture

similar quantities of the reagent and of a saturated aqueous solution of potassium persulphate are added, when indole produces a rose-pink colour.

The cholera spirillum reduces peptone with formation of nitrites, and gives the reaction on the addition of acid alone.

The production of indole depends on the presence of the tryptophan group in the culture medium, and Zipfel has proposed the following medium as most suitable: ammonium lactate (0·5 per cent.), dicalcium phosphate (0·2 per cent.), magnesium sulphate (0·02 per cent.), and tryptophan (0·03 per cent.).

Production of Acid and Alkali.—Many bacteria produce ammonia in a sugar-free broth, while the power of some to ferment sugars, with the production of acid, and sometimes of gas as well, is used for diagnostic purposes.

Nitrification, Denitrification, Nitrogen Fixation.—The bacteria concerned in the nitrogen cycles are dealt with in the chapter on 'The Bacteriology of Soil.'

The Ptomines (Cadaveric Alkaloids).—During the decomposition of proteins, bodies similar in constitution to the vegetable alkaloids may be formed. Some, like methylamine, dimethylamine, and trimethylamine, are non-poisonous; but others, such as muscarin (found in poisonous mushrooms), tyrotoxicon (found in poisonous cheese, milk, and ice-cream), and mytilotoxin (found in mussels), have very toxic properties. It is doubtful whether these products are responsible for so-called ptomine poisoning. Many outbreaks are due to infection with an organism (such as *B. enteritidis*). See 'Bacteriology of meat,' Chapter XX.

Toxins.—Bacteria may give rise to disease in various ways: by appropriation of nutriment, by abstraction of oxygen from the tissues, and obstruction of capillary vessels through their excessive multiplication. Their most important method of aggress, however, is in the production of poisonous bodies, known as 'toxins.' Toxins are supposed to be protein in nature, and in many cases resemble enzymes. They are probably not products of disintegration, like the ptomines, but are specific metabolic products of the bacterial cell.

Toxins may be retained in the bacterial cell as an integral part thereof (endotoxins or intracellular toxins), or, after their formation in the cell, they may be excreted

into the surrounding medium (extracellular toxins). The diphtheria and tetanus bacilli produce extracellular toxins, and, if a fluid culture be passed through a Pasteur filter, the toxins pass through, giving a toxic filtrate. The bacilli of typhoid, anthrax, plague, and cholera, produce endotoxins, which are retained in the organisms in the filter, and the filtrate has but slight toxic properties.

Pathogenesis.—The application of the adjective 'pathogenic' to an organism does not signifiy more than that, given a certain set of conditions in a susceptible host, it is able to produce disease. No sharp line of demarcation can be drawn between the pathogenic and non-pathogenic organisms, for even those regarded as harmless may on occasion produce ill-effects, as when the *B. subtilis* is introduced into the human eye.

Koch's Postulates.—Before an organism can be regarded as specific for a certain disease, it must be shown to conform to certain requirements which have been formulated by Koch:

1. The organism must be present in the tissues, fluids, or organs, of the animal affected with, or dead from, the disease.
2. The organism must be isolated and cultivated outside the body on suitable media for successive generations.
3. The isolated and cultivated organism, on inoculation into a suitable animal, should reproduce the disease.
4. In the inoculated animal the same organism must be found.

To these Hewlett adds—

5. Chemical products with a similar physiological action may be obtainable from the artificial cultures of the micro-organism and from the tissues of man or animals dead of the disease.
6. Specific serum and other reactions, agglutinative, bacteriolytic, complement fixative, etc., are generally obtainable, under certain conditions, if the blood of the infected person or animal be allowed to act on the specific organism producing the infection.

Although an organism fail to conform to all these conditions, other considerations may justify its association with the affection.

Methods of Spread of Infection.—*Contagion* was the term applied to infective matter when contact with a diseased person was supposed to be necessary for the

acquirement of the disease, while if the contaminating matter was conveyed aerially it was known as *infection*. At the present time distal aerial convection is seriously entertained only in regard to smallpox, and even over this disease opinions differ. Now, no distinction is made between infection and contagion, and it is usual to class diseases associated with micro-organisms as 'infective.'

The principal methods of infection are—

1. Pulmonary infection, the bacteria or spores being inspired. Except where the bacteria are protected, as when in moist droplets, infection through the air is not common.

2. Intestinal infection, the organisms being swallowed with food, water, or dust.

3. Inoculation through a wounded or unwounded surface of the skin or mucous membrane.

4. Inoculation through the agency of some biting insect or other intermediate host, a developmental cycle usually, if not always, being passed in the host.

Infection by contagion, fomites, etc., may also occur, in which the manner of entrance of the virus into the body is not precisely understood.

Infection may be restricted to a particular portion of the body (*local infection*), or may be distributed more or less consistently thereover (*general infection*). *Septicæmia* is the term applied to the infection when the organism is carried over the body in the blood-stream, *sapræmia* when the organism is localised and the bacterial products alone enter the system, and *pyæmia* to the development of metastatic abscesses in the liver, joints, lymphatic glands, etc.

Epidemics.—Many diseases are more or less persistent in a restricted locality (endemic). Thus leprosy is endemic in the Sandwich Islands, cholera in the Ganges Delta, and smallpox in the Soudan. On occasion a disease may spread over wide areas, when it is said to be 'epidemic,' or if spreading over the globe, more or less, it is 'pandemic.'

Susceptibility.—The power of an organism to infect is determined by many conditions. Hunger, thirst, excessive fatigue, exposures to extremes of temperature, debility, and immaturity, all predispose an individual to infection. On the part of the organisms many considerations are involved. If subcultured through many generations on artificial media, most become 'attenuated'—

i.e., much of the virulence is lost. A much larger dose will then be required to produce an effect on a susceptible animal, but the virulence may be enhanced by passage through a suitable animal.

The virulence of an organism may be 'attenuated' artificially; for example, by exposing cultures of anthrax to a temperature of 40° C. for some time, they become attenuated to such a degree that they will kill nothing larger than mice.

While many animals exhibit more or less susceptibility to an organism, some may absolutely resist infection therewith (*natural immunity*).

In the case of several diseases, notably in smallpox, to a less degree in measles, mumps, whooping-cough, and scarlet fever, it is not often that the same person is attacked twice by the same disease. That is to say, one attack is 'protective,' and in the above-mentioned diseases the 'protection' may last a lifetime, but extends only to that particular disease, and does not in any way protect against other diseases (*acquired immunity*). On the other hand, an attack from certain other diseases may even predispose the patient to a second attack of the same disease. This is true of influenza, diphtheria, pneumonia, and erysipelas. A distinct predisposition may be caused to attack by other diseases—thus, diphtheria and scarlet fever mutually predispose to one another.

The number of bacteria introduced is important, as a cell or tissue may successfully repel a limited number, but succumb to a greater.

Combinations of infectious diseases are sometimes met with, such as syphilis with gonorrhœa, diphtheria with scarlet fever, and pneumonia with typhoid fever. These are known as 'mixed infections,' but if, as is generally the case, one disease has lessened the immunity to the one acquired last, the latter is known as a 'secondary infection.'

Symbiosis.—*Symbiosis* is the cohabitation of two different organisms for mutual benefit, or their co-operation to produce certain reactions. The presence of streptococci appears to enhance the virulence of the diphtheria bacillus. The effect on animals of an organism may be greatly enhanced by the injection along with it of some other organism that has not pathogenic properties, but which,

in some way that we do not yet understand, adds greatly to the virulence of the pathogenic organism which it accompanies. Attenuated cultures of *B. anthracis* may reacquire virulence if injected simultaneously with a culture of *B. prodigiosus*, and attenuated tetanus bacilli become greatly exalted in virulence when cultivated with the *Proteus vulgaris*.

Streptococci and colon bacilli are not uncommonly concerned in cystitis. When the urine is alkaline, the streptococci attain predominance, while an acid urine suppresses them and allows the colon bacilli to get the upper hand.

Antagonism of Species.—One species suppresses another by exhausting the food material or by excreting metabolic products detrimental to the growth of the other.

Immunity.—Insusceptibility to the attack of a pathogenic organism may be natural to a tribe (*racial immunity*), or to a person (*individual immunity*).

The animal economy stubbornly resists attack by pathogenic bacteria. If any be swallowed, the acidity of the gastric juice will probably destroy them, and the normal flora of the intestine tend to suppress harmful organisms. The nasal secretion entangles and destroys organisms that have been inspired. The tonsillar epithelium acts as a bacterial filter, preventing passage of bacteria at times, but allows free entry into the lymph-channels at others. Wright regards this as a physico-chemical process affecting the surface tension of colloids, of which the cells and bacteria are composed. Even milk, when first drawn, has a germicidal action. When infection takes place through skin or mucous membrane, the phagocytes can dispose of many alien bacteria (see below). Resistance is also offered to toxins, which are destroyed or eliminated by various processes, such work being a notable function of the liver. All these processes are non-specific, with more or less restricted capacity for eliminating bacteria and their toxic products. When, owing to the number of bacteria or amount of their toxins offered, they are overcome, the infection will obtain a hold unless a degree of immunity specific for the particular organism or toxin is available. *Artificial immunity* may be *active* or *passive*.

Active immunity may be produced by one of the following methods:

1. By injection of the virulent organisms in non-lethal doses.

2. By injection of the dead organisms.

3. By injection of the toxic products prepared from filtered broth cultures of the organism.

4. By the injection of the living, but attenuated, organism prepared by one of the undermentioned methods:

(*a*) By passing through a naturally resistant animal.

(*b*) By growth at unsuitable temperatures or in unsuitable atmosphere.

(*c*) By frequent and prolonged subculturing.

(*d*) By growth in the presence of very weak antiseptics.

(*e*) Growth in a medium of unfavourable composition or reaction.

5. By feeding dead cultures of an organism. Achievement of a degree of immunity by this means is slower and less certain than by others. It has been more successful with glucosides such as ricin and abrin, and with snake venom.

An immunised animal is said to be ' protected ' against the specific disease. Protection against one disease sometimes also carries an immunity, though to a less degree, against another.

Passive Immunity.—The serum of a protected animal has an antagonistic effect on the virulent bacteria if injected into a second animal at the same time as, or shortly after, infection. Such immunity is transient. The serum of an animal highly immunised against a particular toxin is properly known as ' antitoxic serum '; that of an animal highly protected against a particular organism in a virulent condition is known as ' antimicrobic,' or ' antiserum.'

To account for acquired immunity the following theories have been adduced:

Exhaustion or Pabulum Hypothesis.—The bacteria are assumed to abstract from the blood some compound necessary for their growth, so that, once this pabulum is exhausted, a second attack cannot take place until it has been re-formed. As an organism will grow in the blood or tissues removed from an animal immune against it, this theory is untenable. Ehrlich, in his *Atrepsy Theory* modifies the hypothesis by assuming the presence of ' chemo-receptors ' for binding the poison before it can act.

The Antidote or Retention Hypothesis.—After the first

attack the organisms are presumed to leave behind them some product of metabolism that is inimical to their existence. A later form of this theory was that antitoxin was a modified toxin, which supposition has been disproved.

The Phagocytosis Theory (Metchnikoff).—The large mononuclear leucocytes and the polymorphonuclear leucocytes ingest and destroy such bacteria as obtain access to the blood-stream. If infection occurs in one locality, there is a simultaneous movement of leucocytes to that spot to cope with the bacteria (*positive chemiotaxis*). When no such attraction takes place, *negative chemiotaxis* is said to occur. Metchnikoff ascribes the process of immunisation to the 'education' of the phagocytes. It is supposed that phagocytes contain digestive ferments (*cytases*) which effect bacteriolysis (solution of bacteria) intracellularly. Or, on the breaking up of the phagocyte (phagolysis), bacteriolysis may be effected extracellularly, and the cytases are then the alexines or complements of Ehrlich's theory. While phagocytosis constitutes a most important factor in the production of immunity, it requires further development to explain passive immunity.

Phagocytosis depends on the presence in the serum of 'opsonins,' which act on the bacteria, and in some way render them suitable for ingestion by phagocytes.

Opinions are divided on the question whether normal blood contains specific opsonins for different diseases, or whether the opsonins are 'common' and act irrespective of the bacteria proffered. However, in the process of immunisation, opsonins of specific character are developed.

Ehrlich's Side-Chain Theory.—Ehrlich assumes that protoplasm is composed of complex molecules possessing very unstable 'side-chains,' ready to combine with other atomic groups if suitable ones come within the sphere of action. In the production of antitoxin the following is assumed to take place: A group on the toxic body (or toxoid), called the *haptophore* group, combines with a group on the cell called the *receptor* group. If the cell is not too much damaged, it produces an excess of receptor groups more than sufficient to combine with the toxin, thus following Weigert's law that continued stimulation is attended by overproduction—hypertrophy. After repeated injections of toxin, so many receptor groups are

formed that the cell cannot hold them, and they become detached, and float about, ready to unite with any fresh toxin introduced. These free receptor cells constitute the antitoxins, agglutinins, etc. Besides the haptophore group, the toxin molecule contains another group called a *toxophore* group, which, if it unites with another group on the cell known as the *toxophile* group, after the union of the haptophore and receptor groups, sets up poisoning. If, however, the toxin come in contact with free receptor cells (antitoxin, etc.), with consequent union of its haptophore groups thereto, it cannot produce poisoning, although its toxophore groups are free, because the first essential necessary, the fixation of the toxin to the cells by the combination of the haptophore and receptor groups, is no longer possible. The toxophore group is more readily destroyed than the haptophore group. By heating a toxin for some time from 140° to 158° F. its toxicity is destroyed, although it still possesses an affinity for antitoxin, because the toxophore group, which conditions poisoning, has been destroyed, but the haptophore group, which unites with antitoxin, is intact.

Production of Antibodies—*Antitoxin.*—When an animal is rendered immune to a toxin a substance known as 'antitoxin' is developed in the blood, which possesses the power of neutralising the toxin.

Aggressins.—Some believe that before infection can take place the bacteria must elaborate additional toxins (aggressins) in the body to overcome the natural resistance. This is presumed to be effected by paralysing the leucocytes and so stopping phagocytosis. Perhaps the aggressins are identical with the true endotoxins of micro-organisms.

Hæmolysins (see p. 20).

Cytotoxins.—The blood-serum of an animal injected with such cells as leucocytes, spermatozoa, ciliated epithelium, or cells from the liver, kidney, or nerves, possesses the power of immobilising and destroying cells of the same origin as those with which the animal was injected. The phenomenon is known as 'cytolysis.'

Agglutinins.—The serum of an animal suffering from, or infected with, typhoid or cholera acquires after a few days the power of causing the aggregation together of typhoid bacilli or cholera vibrios respectively, when these are mixed with it. The *agglutination* is caused by

agglutinins (see Widal Reaction, p. 104, and Saturation Test, p. 152).

A serum made from one organism may agglutinate bacteria of a closely allied species, but in a less marked manner.

Precipitins.—An antiserum produced by the injection of an animal with a substance containing proteins, if added to a solution of the substance with which the inoculation was performed, causes a cloudiness or precipitate. The test is very delicate, especially when performed as a 'ring test' in tiny tubes. It is used for the detection of horse-flesh in sausages, of castor beans in cattle cake, and for ascertaining the species of animal from which a blood-stain came. The sole drawback to the test is the length of time (up to six weeks) necessary to produce an antiserum. Feeble reactions are also obtained with proteins allied to that used in the production of the antiserum.

Antigens.—Any substance which, when introduced into the blood, provokes the formation of antibodies for the defence of the organism is called an *antigen*. Blood-corpuscles, viruses, micro-organisms, etc., are thus all included in the term 'antigen' without prejudging their nature. The *antibody* formed through the introduction of an antigen consists of two bodies. One is 'thermolabile' (destroyed by a temperature of 55° C.), and is termed an *alexin*, *complement*, or *addiment*. The other body is 'thermostable,' and requires a temperature of 75° C. for its destruction. It may be called by various names—*amboceptor*, *sensitiser*, *agglutinin*, or *precipitin*. While the alexin is present in the serum of every animal, whether healthy or diseased, the amboceptor is specific, being peculiar to a particular antigen.

Hæmolysis.—If blood-corpuscles be injected into an animal, the blood of the latter acquires hæmolytic properties, and dissolves red corpuscles of the same origin as those which were injected. The solvent agent produced ('hæmolysin') contains a *complement* and an *amboceptor* or *immune body*. For *Hœmolysis Test* see p. 152.

Fixation or Absorption of the Complement.—Cholera-immune serum, inactivated (*i.e.*, the complement is destroyed) by heating to 56° C. for half an hour, is mixed with the cholera vibrio, when after a time the vibrios are

found to have absorbed the immune body. The test can be employed for other organisms and for red blood-corpuscles.

The same complement will sensitise either hæmolytic or bacteriolytic immune bodies. A mixture of typhoid bacilli, inactivated typhoid-immune serum, and guinea-pig serum, is incubated at blood-heat for two hours, then 'sensitised' corpuscles (*e.g.*, a mixture of inactivated serum hæmolysing sheep corpuscles *plus* washed sheep corpuscles) are added and incubation continued for a further two hours. No hæmolysis occurs. The bacillary amboceptor has absorbed all the guinea-pig complement, leaving none to activate the amboceptor of the serum hæmolytic for sheep corpuscles. Consequently no hæmolysis of the sheep corpuscles is possible (*Bordet and Gengou phenomenon*). Should normal serum (inactivated, of course) be used instead of typhoid-immune serum, there is no amboceptor to absorb the guinea-pig complement, and the latter is available for absorption by the inactivated hæmolytic serum, and hæmolysis occurs.

The following test is based on this phenomenon:

Wassermann's Test (*Fixation Test, or the Antigen Test*).—A guinea-pig or rabbit is inoculated several times intravenously with the washed blood-corpuscles of a rabbit or sheep, with the consequent production of a hæmolytic serum specific for the corpuscles of a rabbit or sheep respectively, and the serum is inactivated. An *antigen* is prepared by mincing and triturating the liver of a syphilitic fœtus in physiological salt solution. The serum from the patient (the *test fluid*) is inactivated in the same way as the hæmolytic serum—by heating to 56° C. for thirty minutes, thus destroying the alexin. A *complement* is made by diluting guinea-pig serum tenfold. The test fluid is added to the antigen extract, some complement is added, and the mixture left for four hours at 20° C. The *hæmolytic system* (a mixture of inactivated hæmolytic serum and the washed blood-corpuscles for which it is specific) is added, and if after four hours no hæmolysis has taken place syphilitic taint is present. The antibody in the patient's blood has attacked the treponemes which abound in the liver of the infected fœtus, the complement is absorbed, and there is none left to cause the inactivated hæmolytic serum to dissolve the blood-corpuscles.

Conversely, if no syphilitic antibody exists in the patient's blood, the complement is left free, and is *deviated* to the sensitiser of the hæmolytic serum, and allows this to cause hæmolysis of the blood-corpuscles.

Controls with normal and with syphilitic sera with and without antigen are put through at the same time. Cultures of the treponemes do not prove suitable antigens, but various other substances act as well as the liver of a syphilitic fœtus—*e.g.*, alcoholic extract of normal heart-muscle + cholesterin. For this reason the reaction is not a true antigen test. (See also p. 182.)

Bacteriolysis and Antimicrobic Sera.—If an animal be treated with gradually increasing doses of an organism, an immunity against this organism is, to a certain extent, created. If a mixture of the animal's serum with the bacteria be injected into an animal, subsequent examination shows the bacteria in a state of dissolution (*bacteriolysis* or *Pfeiffer's phenomenon*). In *Pfeiffer's reaction* this solution of bacteria is applied to determine the species of an organism, particularly of the cholera vibrio. Two milligrammes (a loopful) of an eighteen or twenty-four hour agar culture of the *virulent* isolated vibrio is suspended in 1 c.c. of broth containing 0·001 c.c. of the serum of an animal very efficiently immunised to cholera. The mixture is injected into the peritoneal cavity of a 250 gramme guinea-pig. After intervals of thirty and sixty minutes, some of the peritoneal fluid is abstracted by a sterile capillary pipette, and a hanging-drop preparation made therefrom. If the organism be *V. choleræ*, the bacteria are seen broken down into granules. A control experiment with normal serum is made.

The bodies bringing about this phenomenon are known as *bacteriolysins*. Bacteriolysis is brought about by two substances, an *immune body* different for each organism, and only existing subsequent to treatment with the specific organism, and an *addiment*, *complement*, or *alexin*, present in normal serum. The complement is highly unstable, and present in small amount, thus restricting the curative power of antityphoid and anticholera sera.

Deviation of the Complement.—An excess of immune body in a serum proves as inefficient for bacteriolysis as too small a quantity. Some amboceptors unite with the receptors of the bacterial cells, while others combine with

the complement. No free complement is left to combine with those amboceptors that are attached to the bacterial cells. The complex (amboceptor + complement + organism) that is necessary for bacteriolysis is therefore not provided.

Serum-Therapy.—By repeated injection of animals with gradually increasing doses, sublethal at first, of either the specific toxin or the living culture, a state of gradually increasing resistance is acquired by the animals against the toxin or the microbe. The blood attains an immunising power that is transferable to a new subject. If injected into a fresh animal, the blood confers on the latter resistance against the specific infection. The immunising power of such blood-serum comes into action even after infection has already taken place—that is to say, the blood-serum has a curative therapeutic action.

Bacterial Vaccines.—The injection of sterilised cultures of certain pathogenic organisms serves to protect against the diseases concerned. Such vaccines may be administered as prophylactics in anticipation of exposure to the specific infection; or they may be used as therapeutic measures after infection has taken place. Inasmuch as slightly different organisms of the same species may be involved in different cases of an infection, a vaccine is usually made from perhaps ten different strains (*polyvalent vaccine*). A vaccine is specific—*i.e.*, only likely to suppress or cure infection when this is produced by an organism of a character identical with that used for the vaccine. By use of a polyvalent vaccine the probability that the article specific for the infection is used correspondingly increases. A vaccine supplied from organisms isolated from previous cases of the disease (or of other diseases due to the same organism) is known as a *stock vaccine*. If prepared from cases occurring in the same institution or neighbourhood, it is produced from strains of *local flora*. When infection of a case has already taken place, and the responsible organism is isolated, a vaccine may be prepared from it (*autogenous* or *personal vaccine*).

A description of the preparation of an autogenous vaccine will show the principles employed. The responsible organism is identified by microscopical examination of the material obtained, this being supplemented if possible by cultural and agglutination experiments. It is isolated

on appropriate medium, and three or four 'streak' or rather 'slant' cultures made, the whole of the surface of the slanted medium being used. About 1 c.c. of sterile salt solution (0·1 per cent.) is poured into one of the tubes, and by rubbing up with a sterile platinum loop the growth is detached and forms a milky emulsion. This is poured into a sterile test-tube or flask and the culture tube is rinsed out with a few more drops of the salt solution. The process is repeated with the other slants, and their emulsions added to the first, so that a total volume of about 5 c.c. is obtained. The masses present in the emulsion have to be thoroughly broken up, and the bacterial content ascertained by Wright's method: A small definite volume of the emulsion is mixed with an equal volume of blood, and smears made on slides are stained with one of the blood-stains. The relative numbers of red cells and bacteria are determined. Human blood, if from a male, contains five million red cells per cubic millimetre, or a thousand times this number per c.c. A calculation therefore gives the number of bacteria per c.c. The emulsion is diluted to a strength suitable for administration, with sterile normal saline containing 0·5 per cent. carbolic acid, and sterilised for an hour or an hour and a half in a water-bath at 56° to 60° C. This will not always suffice for sterilisation. In such cases a further sterilisation at 60° C. for one hour, twenty-four hours later, is to be preferred to a single sterilisation at a higher temperature. With some of the cocci a temperature of 65° or even 75° C. is necessary. Prolonged heating or the use of too high a temperature lowers the activity of the vaccine. Before use a subculture must be made from the vaccine to prove its sterility, and all through the process of preparation rigorous sterility of apparatus, diluting solution, etc., must be maintained.

A main cause of failure in vaccine treatment lies in the selection of the wrong organism. This is liable to occur with the colon bacillus, of which there are said to be 150 varieties, so that the infecting organism may not be matched even in a polyvalent vaccine. It is often difficult, sometimes impossible, to say at a first examination which of the organisms found is the ætiological agent, and when a vaccine fails a further examination is necessary. Perhaps two or three bacteria are responsible, and the vaccine has only been prepared from one, with the result that the

others carry on until a mixed vaccine is discovered to be necessary. Success in vaccine-therapy also depends on the correct dosage and the observance of a right interval between doses.

Anaphylaxis.—Instead of an injection rendering the subject less sensitive to further injections of the same substance, the reverse sometimes occurs, certain poisons creating a peculiar sensibility on the part of the organism towards themselves under conditions that would lead one to anticipate a tolerance. Delille defines anaphylaxis as 'a state of acquired vulnerability in an organism to a second injection of a substance to which, at the time of its first injection, it was indifferent.' For the production of anaphylaxis an interval (the latent or incubation period) must elapse between the first or sensitising dose and the second or reacting dose. The minimum length of time is said to be ten days, and should the second dose be administered within this time anaphylaxis will not develop. The length of time for which a sensitising dose will remain effective in increasing sensibility is not known.

Anaphylaxis is in some measure specific—*i.e.*, the second injection must be of the same nature as the first. While it has been mainly studied by the injection of horse serum into guinea-pigs, the phenomenon has been obtained with proteins, toxins, animal sera, glycogen, peptone, trypsin, saponin, sodium oleate, in fact it is asserted that any colloid will induce anaphylaxis. While crystals do not produce the reaction, quinine, antipyrin, and iodoform are apparent exceptions, and in some predisposed persons inevitably lead to urticaria and anaphylactic sickness.

It is necessary that the substance injected be foreign to the animal used, and that the same or an allied substance be employed for the reacting as for the sensitising injection. A guinea-pig sensitised with horse serum will not react if the second injection be sheep serum or goat serum, but it should react if the second injection be donkey or mule serum.

Animals injected with a particular organism are anaphylactised by the corresponding toxin in a strictly specific manner. Curious to say, it is necessary that the sensitising and the exciting dose must take place through the same route.

Anaphylaxis *in vitro* may be induced by adding horse

serum to the serum of a rabbit sensitised to horse serum, in a test-tube. If immediately injected into a rabbit anaphylactic symptoms, and perhaps death, follow. So small a dose as a millionth part of a c.c. of horse serum will sensitise a guinea-pig. If after a period of incubation a second dose be given, the symptoms of the so-called anaphylactic shock appear. Death may occur almost immediately; if not, the animal becomes restless, falls over, and after diarrhœa, convulsions, and respiratory failure, paralysis follows. Death may occur in this state or rapid recovery may follow (Theobald Smith phenomenon). The hypersensitive state may be transmitted by the female guinea-pig to her progeny. The blood of anaphylactised animals, if injected into normal animals, confers anaphylaxis after a large number of injections, sometimes, indeed, after a single injection (passive anaphylaxis).

No satisfactory explanation of anaphylaxis has been made. Gay and Southard consider that the serum contains a substance provisionally termed anaphylactin, which remains as a constant irritant to the cells of the body, increasing their reactivity for the other constituents of the foreign serum. Vaughan and Wheeler believe that anaphylaxis must be due to a toxic fragment of the protein molecule. Richet thinks that in the injected animal there is produced a substance (*toxogenin*) which is not toxic in itself, but yields a toxic substance (*apotoxin*) on combination with antigen. The toxicity of the apotoxin (or precipitin) is increased by combination with the alexin of the blood.

In the serum the precipitin content runs parallel with the severity of the symptoms, and disappears after the anaphylactic condition passes off. Halliburton has suggested that this explains the difference between the normal and the sensitive animal.

The frequency with which normal horse serum and antidiphtheritic serum are used in treatment, proves anaphylactic shock to be of rare occurrence among human beings. Some workers question if death ever occurs, outside of cases of status lymphaticus.

Serum Disease or Serum Sickness.—About one-third of persons injected with horse serum for the first time are found to be sensitive to it, an urticaria, more or less œdema, and sometimes arthritis, appearing between the sixth and

twentieth days after injection. The only explanation offered is that part of the serum used as an injection remains unaltered in the subject, while the remainder sensitises the serum of the subject. In fact, a sort of auto-anaphylaxis occurs.

Snake Venom.—The venoms of different species of poisonous snakes differ greatly in composition. Some appear to contain proteolytic enzymes which are supposed to produce the softening of the muscles in the animals attacked. Cobra venom contains a hæmolysin, innocuous by itself, but activated by normal complement in the blood-serum of the victim. This hæmolysin is also activated by lecithin.

Rattlesnake venom acts partly by lysis of the endothelial lining of bloodvessels, the specific toxin from its effects being called 'hæmorrhagin.' Other venoms, such as that of the krait, produce intravascular thrombosis through an almost instantaneous coagulation of the blood. Many venoms are neurotoxic, the neurotoxins of different species selecting different sites for activity, one acting on the respiratory centre, another on the nerve endings in muscle. Serpent venom, unlike true bacterial toxins, is unaffected in virulence by a considerable degree of heating.

Antivenin or Antivenomous Serum.—Calmette injects horses with gradually increasing doses of cobra venom mixed with diminishing quantities of a 1 in 60 solution of hypochlorite of lime. When they have acquired sufficient immunity, the venoms of as many species of reptile as possible are injected. The process of immunisation lasts at least fifteen months.

Calmette's serum is active to the extent of 1 to 200,000—that is to say, it is sufficient to inject as a prophylactic dose a quantity of serum into a rabbit equal to $\frac{1}{200000}$ of its body-weight; 0·5 c.c. of this serum is sufficiently active to protect a rabbit against a dose of venom, which otherwise would be lethal in three or four hours, if it is not injected later than half an hour after the bite. The dose of the antivenin for a human being is, according to Calmette, 10 to 20 c.c., but Lamb and Hanna consider that it should be 30 to 40 c.c., injected as soon after the bite as is practicable.

It has been asserted that cobra antivenin protects animals against any snake poisons; but Martin and

Tidswell have shown that antivenomous serum is just as specific as other antisera.

Bacterial Mutability.—The production of involution forms (p. 3) and of attenuated bacteria (p. 14) have been already dealt with. Appearance in different forms at different times—*e.g.*, as a bacillus and leptothrix—is called *pleomorphism.*

Bacteria that normally are 'acid-fast' or 'Gram-positive' may fail to resist decolorisation by acid in the Ziehl-Neelsen process, or may become 'Gram-negative,' when old. This is most noticeable among the *Streptothricæ.*

Other changes in character occur especially in the colon-typhoid group. Twort endowed a strain of typhoid bacilli with lactose-fermenting power. Revis states that the fermentation of a sugar or polyhydric alcohol takes place in two stages—a preliminary acid formation and a subsequent gas formation. Revis succeeded in causing an organism to lose its power to produce gas while retaining its capacity to produce acid, the resulting variety being of a permanent character.

It should be noted that an organism may ferment a specific carbohydrate obtained from one dealer, and have no action on the same substance as supplied by another.

By exposure to ultra-violet rays Mme. Victor Henry converted anthrax bacilli into cocci and other forms which are apparently stable. It also appeared to lose its capacity for secreting proteolytic enzymes.

Simonini found thorium salts to modify morphology, staining reactions, and physiological characters of Shiga-Kruse and Flexner dysentery bacilli and *B. diphtheriæ*, *B. coli* showing less response.

Theile and Embleton showed that a guinea-pig previously sensitised to *B. mycoides* died after inoculation with this organism, and the *post-mortem* appearances were indistinguishable from those of anthrax. After passage through the sensitised animal, the organism was capable of producing disease in a normal animal. Similarly, smegma and Timothy-grass bacilli produced in animals specifically sensitised *post-mortem* appearances indistinguishable from those due to an intraperitoneal injection of tubercle bacilli.

Dostal claims to have converted the tubercle bacillus into a non-acid-fast and non-pathogenic organism.

CHAPTER II

BACTERIOLOGICAL APPARATUS

The Microscope.—A microscope for bacteriological work should be absolutely rigid, and the fine adjustment should be sensitive and precise. It must be fitted with a suitable substage condenser, with an arrangement for focussing, and iris diaphragm. A triple nose-piece avoids unscrewing the objectives to obtain variations in power, and saves not only time, but much wear and tear. The following objectives are required: $\frac{2}{3}$-inch, $\frac{1}{6}$-inch, and a $\frac{1}{12}$-inch oil immersion. These objectives combined with an 'A' or 'B' eye-piece will give magnifications to 1,100 diameters, which is ample for all ordinary purposes.

A mechanical stage of an easily detachable form is desirable, especially for the systematic examination of blood-films, etc.

A brilliant illumination is essential for the examination of bacteria, particularly when in tissues. A paraffin lamp with flat flame, the *edge* of the flame being used, is very satisfactory; or, if electric current be available, a Nernst lamp enclosed in a frosted globe, or the Barnard lamp. Daylight is not always suitable, but it is as well to be able to use it on occasion.

Micro-organisms in liquids and tissues are only visible through the shadows caused by the differences in the refractive power of the various structures. Consequently the hole in the diaphragm must be diminished. In the case of stained specimens, however, an open diaphragm can be used, and the preparation examined with the full aperture of the condenser.

After using the oil-immersion objective, the cedar oil should be removed with soft filter-paper and the lens then wiped with a silk handkerchief. Should the oil be allowed to dry on at any time, a little fresh oil should be put over it and allowed to stand a short time; this will soften the hardened oil, when the whole may be cleaned off together, or it may be gently cleaned with a rag moistened with xylol.

Limits of Microscopical Vision.—There is a limit to the visibility of microscopical objects. With the very best optical appliances and the use of monochromatic violet light it is impossible to see more than about 167,000 lines to the inch, an object measuring less than about 0·14 μ not being perceptible. By means of transverse illumination, ultra-microscopic particles may be rendered visible as diffraction discs, and particles measuring far less than half a wave-length of light can be made visible (Siedentopf's apparatus).

The Hot-Air Steriliser.—An iron box, with double walls, fitted with a door in front and supported on four legs. It is heated by means of a rose gas-burner from below, and the temperature of the interior is indicated by means of a thermometer inserted through a hole in the top. If necessary, a mercury-gas regulator may be inserted through a second opening.

The temperature in these ovens is by no means uniform; it therefore must be ascertained that the objects exposed for sterilisation really reach the desired temperature.

Test-tubes, Petri dishes, pipettes, etc., may be thoroughly sterilised by exposure to a temperature of 150° C. for one hour. The door must not be opened until the temperature has dropped to 60° C. Neglect to observe this precaution courts cracked glass. Inoculating-wires, forceps, etc., are best sterilised by passing through the flame of the Bunsen burner.

The Steam Steriliser.—This is a cylindrical vessel of copper, about ½ metre high by about 30 centimetres wide, jacketed with non-conducting material, and provided with a lid. The lid is covered with felt, and is perforated to receive a thermometer. Inside the vessel is a diaphragm or grating about two-thirds down which divides the interior into two portions: the upper, or 'steam-chamber,' and the lower, or 'water-chamber.' A water-gauge indicates the water-level. The apparatus stands upon three legs, and is heated by a large Fletcher burner, keeping the water in vigorous ebullition, so that steam issues freely from the top. A uniform temperature of 100° C. is thus maintained in the apparatus. The steriliser is fitted with a wire basket or metal rack for the reception of test-tubes containing nutrient media.

This apparatus is employed for sterilising media and

apparatus which cannot be exposed to temperatures above 100° C. Glass utensils may be steamed for from one to two hours.

The High-Pressure Steam Steriliser.—High-pressure steam in an autoclave acts with greater rapidity than ordinary steam. Although not necessary for ordinary use, in the sterilisation of soil it must be used. Certain spores resist ordinary steaming for three hours, but are destroyed in fifteen minutes by steam at 110° to 120° C.

Sterilisation by Chemical Agents.—For washing instruments, and for disinfecting the hands, solutions of 1 in 1,000 of corrosive sublimate, 1 in 20 solution of phenol, or 1 in 50 solution of lysol, are used. When chemical agents are used, risk is incurred by traces of the germicide escaping removal, and destroying the organisms under examination or introducing other elements of uncertainty into the work. For ordinary purposes it is best to rely upon the careful fulfilment of all the details required in the sterilisation by the usual methods.

Glass pipettes, etc., may be rapidly sterilised by rinsing with 5 per cent. phenol, then with absolute alcohol, and lastly with ether, the ether finally being driven off by careful heating over the Bunsen.

Steel instruments, etc., are best boiled in water containing a little sodium carbonate.

Sterilisation by Filtration.—Air and other gases are readily freed from micro-organisms by drawing them through a tube containing a plug of dry sterile cotton-wool or packed with sugar or sand.

Water or other liquid which is not too viscid is sterilised by passage through unglazed porcelain cylinders (Pasteur-Chamberland filter). These filters are used for the purposes of concentration of bacteria in a liquid, or the separation of bacteria from their products.

For experimental purposes these filters must be cleaned and sterilised before being used. This operation does not affect the Pasteur filter, but tends to disintegrate the Berkefeld (see p. 239).

The Microtome.—A large number of machines for the cutting of sections of tissues have been introduced. For some the tissue is frozen before cutting, for others it is first impregnated with paraffin or celloidin.

The Incubator.—The pathogenic bacteria grow best at the temperature of the body of the host, and for their culture an incubator with a temperature of 37° C. is employed. Many of the saprophytic forms will not develop at so high a temperature, and they are cultivated either in a warm room or in a 'cool' incubator, at about 22° C. The incubator consists essentially of a double-walled chamber, the space between the walls being filled with water warmed by a gas-burner. The outer wall is covered with some non-conducting material. In the Hearson incubator a regular temperature is secured by an Excelsior gas-valve, in which the pressure of ether and other vapours is employed in a flexible envelope; this, acting upon a lever, controls the gas-supply. Page and Reichert thermostats are also used, where a fall in temperature occasions the contraction of mercury and allows more gas to pass, while if too hot, mercurial expansion ensues, and by partially cutting off the gas, diminishes the flame. In case the main gas opening should become closed by the expansion of the mercury, a by-pass allows the maintenance of a pilot light.

The 'cool' incubator is similar in principle, but is surmounted by a vessel containing ice. The regulation of temperature within the chamber is effected by a small stream of water, which runs continuously through the apparatus in one of three directions, the choice being automatically determined by a thermostatic capsule. A third incubator, giving a temperature of 42° C., is useful for the culture of typhoid and colon bacilli in water, etc., examinations.

Centrifuges.—For the removal of fine particles from suspension, centrifugal force is employed. The Gerber machine, as used in milk analysis, when fitted with centrifuge tubes, answers well, and special machines are made for the purpose. For small quantities of material, hæmatocrites are used.

Inoculating Needles.—For the transference and spreading, etc., of material, pieces of platinum wire fused into glass, or fixed into aluminium rods, are used. For ordinary purposes 0·4 millimetre (27-28 B.W.G.) wire is suitable, but the greater stiffness of a 0·7 millimetre wire is sometimes necessary. Needles, both straight and with loops (of varying sizes up to 4 millimetres in diameter or bigger) are used.

Test-Tubes.—$6 \times \frac{5}{8}$ inches is the most useful size. For special purposes smaller and larger ones are required.

Cornet forceps for cover-glasses, dissecting forceps of various patterns, and other instruments, are needed, together with flasks for holding media.

CHAPTER III

THE PREPARATION AND USE OF NUTRIENT MEDIA

It is necessary, in order to obtain a satisfactory knowledge of the biological characters of a micro-organism, to obtain a pure culture—that is, a culture containing one species only.

When, by exposure to air or by other means, isolated bacteria lodge on the surface of a nutrient medium, they are fixed *in situ* and commence to grow, each organism producing a colony which eventually becomes visible macroscopically. This character is used for the *isolation of organisms.* Pure cultures of bacteria are hardly ever met in practice, and a very common method of separating individual bacteria is to disperse the liquid containing them over or through some solid medium in such a dilution that individual bacteria can form sufficiently large colonies without their meeting. This is generally effected in Petri dishes.

By the introduction of a part of a colony into a tube of sterile medium, a pure culture is obtained after incubation, and the larger quantity of growth provides more material for examination. The appearances of the growths on various media constitute important, and often positive, means of identification.

In culture, the store of nutrient material becomes gradually used up, and reproduction stops. It is necessary, therefore, to reinoculate them from time to time into fresh media. Bacteria are artificially cultivated in both liquid and solid culture media.

Nutrient media are employed in test-tubes, small conical (Erlenmeyer's) or other flasks, or Petri dishes. All test-tubes, flasks, etc., are thoroughly cleansed with 25 per cent.

hydrochloric acid, and well rinsed with water to remove all traces of acid. The tubes are then allowed to drain until nearly dry, when they are finally rinsed out with a little strong alcohol, drained, and allowed to dry. Or the tubes may be cleaned by boiling in water containing soap powder for thirty minutes, followed by cleansing with brush and thorough rinsing with water. They are then plugged with sterilised cotton-wool, and sterilised for an hour at 140° to 150° C. in the hot-air steriliser. Cotton-wool is sterilised by pulling loosely apart and heating for an hour at 145° C. in the hot-air steriliser. Though sometimes desirable, it is not always necessary to sterilise tubes and cotton-wool before filling with media. The sterilisation after tubing is generally sufficient.

Reaction of Media.—A reaction slightly acid to phenolphthalein (equivalent to a faintly alkaline reaction to litmus) is generally most suitable.

Standardisation of Media.—Variations in reaction of media influence the character of the growths. For the enumeration of organisms and for descriptive work standard media are necessary. The signs + and − indicate acidity and alkalinity to phenolphthalein respectively. The American Committee describe + 1·5 per cent. reaction, when to every 100 c.c. of medium neutral to phenolphthalein 1·5 c.c. of normal hydrochloric acid are added. English workers, following Eyre, use an acidity of +1·0 per cent. (+10 on Eyre's scale).

The procedure is briefly as follows: An aliquot portion of the nutrient medium is taken, say 20 c.c.; this is diluted with warm distilled water, boiled for a minute, a few drops of a solution of phenolphthalein are added, and *decinormal* solution of sodium hydrate is run in drop by drop from a burette to the hot solution until a pink coloration is obtained. The correct volume in c.c. of *normal*, or, better, *dekanormal*, soda solution to be added to the bulk is calculated and added; the reaction of the medium will then be neutral to phenolphthalein, but strongly alkaline to litmus. The alkalinity is too great for the optimum growth of most organisms, and it is reduced by the addition of normal hydrochloric acid to the extent of 1 c.c. per 100 c.c. of medium. The reaction is then said to be + 10 (Eyre's scale) or +1·0 per cent. (American scale).

Instead of first neutralising and then adding normal

acid or alkali, sufficient alkali may be added to *reduce* the reaction to the required point. After standardisation, the medium is heated on a water bath for half an hour to bring down the precipitate caused by the change in reaction (magnesium ammonium phosphate, Jordan), and then filtered.

Preparation of Beef Broth.—One pound of beefsteak, freed from fat and connective tissue, is cut up and passed through a mincing-machine. The finely-minced meat is digested with 1,000 c.c. of water. It is then boiled, with constant stirring, for thirty minutes in a tinned or enamelled saucepan, which is kept well covered. The broth is strained through muslin, and then made up with distilled water to 1,000 c.c., to replace that evaporated during the boiling. This is the ' acid-beef broth.' To the broth is then added 5 grammes of sodium chloride and 10 grammes of peptone. The latter is first rubbed up with a little of the broth in a glass mortar, after which it is added to the bulk.

The mixture is now boiled for five minutes, and then very carefully neutralized with a solution of sodium carbonate or hydrate, making the solution very faintly alkaline to litmus-paper. The alkaline solution is added carefully, drop by drop, shaking the flask well between each addition. The solution is again boiled for ten minutes, with constant agitation. The reaction is again tested with litmus-paper, and if still faintly alkaline, the solution is filtered into a flask through a double-pleated filter-paper. Or the broth may be standardized to + 10 (*vide supra*). The filtered product, which should be absolutely clear and bright, is then run into flasks (which are plugged with sterile cotton-wool) and sterilised on three successive days in the steamer for fifteen to twenty minutes on each occasion. Bullock's heart or sheep's heart may be used instead of steak.

Lemco Broth.—Although some workers prefer beef broth, one in which extract of meat is used serves equally for most purposes, and is now generally used. Lemco, 20 grammes; peptone, 20 grammes; salt, 10 grammes; distilled water, 1 litre. Boil for thirty minutes, standardise, and filter. This is more easy to prepare, and varies less in composition, than beef broth.

If, in spite of filtration, the broth remains turbid, the white of an egg is added to the cooled broth (50° C.),

well mixed, and raised and maintained at the boiling-point for ten minutes. The precipitated albumin is then filtered off, and the filtrate sterilised as above directed.

Stitt uses 3 grammes of Lemco to the litre, and, as the medium has almost invariably a reaction of +0·75 (American scale), considers it is usually unnecessary to titrate and adjust the reaction unless precision is demanded.

Glycerin Broth.—Five c.c. of glycerin to every 100 c.c. of beef broth.

Glucose and Lactose Broth.—To each 100 c.c. of broth is added 1 to 2 grammes of pure glucose or lactose. Used in the cultivation of anaërobic bacteria.

Nutrient Gelatin.—To 1 litre of acid beef broth are added 100 grammes of 'gold label' gelatin, 10 grammes of peptone, and 5 grammes of salt. The mixture is placed on a water-bath until solution is complete, and then rendered faintly alkaline to litmus-paper with sodium hydrate solution. After cooling to 50° C., the white of an egg is added, and after stirring it is steamed for one hour. The gelatin is now filtered through a pleated filter in a hot-water funnel, and then run into test-tubes. The tubes are sterilised on three successive days in the steam steriliser for fifteen minutes on each occasion. After the final steaming they are allowed to solidify in upright or slanting positions, according as to whether they are intended for stab or streak cultivations. In hot weather 15 or 20 per cent. gelatin should be used. The gelatinising power of gelatin is gradually destroyed by heating.

Glucose Gelatin.—Two per cent. of glucose in nutrient gelatin.

Nutrient Agar.—Fifteen grammes of powdered agar are well boiled with a litre of nutrient broth for two to three hours until dissolved; the water lost by evaporation is replaced from time to time. Care is then taken to see that the medium is faintly alkaline, after which it is cleared with egg-albumin, as described under the preparation of gelatin. The agar is then filtered through 'Chardin' filter-paper or a small jelly-bag. Some workers allow the hot agar to stand in the steam steriliser in a tall, cylindrical vessel till the flaky particles which cause the turbidity sink to the bottom, when the clear agar can be poured off.

Agar jelly remains solid at 40° C., and only melts completely at 99° C.; hence this medium is well adapted for the higher incubating temperatures. Nutrient agar is often quite clear when hot, but is always slightly opalescent on cooling.

Glycerin Agar.—Nutrient agar containing 5 per cent. of glycerin.

Glucose Agar.—The addition of 1 to 2 per cent. of glucose to nutrient agar is useful for the cultivation of anaërobic bacteria. The tubes for this purpose are filled two-thirds full. The medium should not be heated more than is absolutely necessary during preparation and sterilisation, or it becomes dark.

Urine Gelatin and Agar.—Fresh urine thickened with 10 per cent. of gelatin, or 2 per cent. of agar, with the addition of 1 per cent. of peptone and ½ per cent. of sodium chloride, is rendered feebly alkaline and filtered.

Peptone Water.—Ten grammes of peptone and 5 grammes of sodium chloride are dissolved in 1,000 c.c. of distilled water; the solution is then well boiled, and neutralised carefully in the usual manner. The solution is again boiled and filtered. The solution is then run into tubes, and steamed for fifteen minutes on three successive days.

Glucose and Lactose Peptone Waters.—The addition of 1 to 2 per cent. of glucose or lactose to peptone water is very useful when determining the fermentative power of organisms. The medium may be tinged with litmus, in order to show production of acid or alkali, sufficient of a neutral solution of litmus being added for this purpose. These media and their corresponding broths are preferably introduced into Durham's tubes, which consist of the ordinary test-tubes into which small inverted tubes have been introduced. The small tubes during the process of sterilisation become filled with the medium, and then serve as gas-holders, should the sugar be fermented with the production of gas. If the inner tubes do not fill during sterilisation, two grease-pencil marks should be made on the outer tube to show the volume of air left in, or the Durham tubes may be put in a water-bath and heated to boiling for ten minutes, when the air bubble will disappear. As a general rule the production of gas can be observed without using an inner tube, as a few small

bubbles are seen at the surface, or can be seen rising in the liquid if the tube is gently shaken. As gas production may have stopped at the time of inspection, it is never wise to dispense with the inner tube.

Maltose, galactose, arabinose, raffinose, cane-sugar, mannite, sorbite, dulcite, adonit, dextrin, starch, and inulin, are similarly used.

Milk.—Fresh separated milk, free from preservatives, is sterilised for twenty minutes on each of five successive days. The medium is frequently tinged with litmus.

Milk must not be overheated, as this retards or prevents the formation of clot when organisms that should produce clotting are grown in it. This, perhaps, is the reason why some brands of non-sweetened condensed milk prove unsatisfactory for milk-tubes. Milk is always so greatly contaminated to start with that it is unwise to rely on sterilisation, and tubes should be incubated at blood-heat for two days in order to ascertain which are sterile.

Potato-Tubes.—Large sound potatoes are thoroughly scrubbed, and then with a large cork-borer cylindrical pieces are cut to fit into test-tubes. Each cylinder is cut into halves diagonally, the wedges are well washed in running water for an hour, and each wedge is placed in a test-tube. The cores of potato rest on a moist plug of cotton-wool to keep the potato cylinder moist. The tubes are capped, and sterilised in the steam steriliser for thirty minutes on each of three successive days. The tubes are left in the blood-heat incubator overnight, and any contaminated ones rejected.

Blood-Serum.—Blood from the jugular vein or an incised wound is allowed to run into a tall sterile glass vessel, with aseptic precautions. The vessel is at once placed in a cool place without the least shaking, and allowed to stand overnight, when a firm clot forms; the clear serum is drawn off by a sterile glass siphon or large pipette into sterile test-tubes, which are plugged, and laid on a slanting surface, and the serum made to set by heating in the hot-air steriliser to 65° C. The tubes can then be sterilised in the usual way by steaming on three successive occasions. All tubes should be tested for sterility by a trial incubation before use. The serum should have a jelly-like consistency, and an opalescent, yellowish-white colour.

Chloroform is particularly suitable for the sterilisation of blood-serum, as it has a powerful germicidal action combined with a low boiling-point, so that it can be driven off with certainty after sterilisation is complete. (The liquid under treatment is shaken up with chloroform, and allowed to stand some days, when it is freed from chloroform by prolonged heating at 62° C.)

The serum from human blood, obtained at operations and from placentæ, occasionally presents advantages over that obtained from animals.

Modifications of Blood-Serum.—The fluid obtained from hydroceles, cysts, or dropsical effusions, is practically the same in composition as blood-serum.

Löffler's Medium.—Two parts of blood-serum with one part of nutrient glucose broth. The medium is solidified in a slanting position.

Blood-smeared Agar.—The surface of the agar in sloped agar-tubes is smeared with blood obtained aseptically. The tubes must not be sterilised after the blood has been added.

Egg-Albumin.—The albumin from birds' eggs is carefully separated from the yolk, and treated as directed under the preparation of blood-serum tubes. The white from plovers' eggs yields an almost transparent medium. Hens' eggs may (Hueppe) be themselves used. New-laid eggs are washed in sodium carbonate solution, immersed in 1 in 2,000 mercuric chloride solution for a short time, thoroughly rinsed in water that has been well boiled, and finally rinsed in strong alcohol and ether. The end is pierced with a sterile needle, and the material to be inoculated is introduced into the egg by means of a glass capillary tube, from which it is blown with great care. The hole is now closed with sterile cotton-wool. This method of cultivation is particularly well adapted for the cultivation of the anaërobic bacteria.

Beer-Wort.—Unhopped beer-wort is allowed to stand in a cool place for twelve hours, filtered, steamed for one hour, again filtered, and then sterilised.

Beer-Wort Gelatin.—One hundred grammes of gelatin are dissolved in a litre of unhopped beer-wort, clarified and filtered, but not neutralised.

Silica Jelly.—This preparation is destitute of organic matter, and is used for the organisms of 'nitrification,'

which will not grow on an organic medium The gelatinous consistency is obtained by means of dialysed silicic acid.

Irish Moss Jelly is used for the culture of the thermophilic organisms, and various other media are employed, the composition of which is given in different monographs.

To prevent evaporation, and assist exclusion of aerial organisms, culture-tubes may be covered with rubber caps, or after the last steaming their mouths may be protected with gutta-percha tissue. Media should be kept in a cool, dark place, such as in suitable-sized tins with lids. A piece of filter-paper may be placed at the bottom of the tin, and a few drops of clove oil sprinkled thereon. In this way the percentage of tubes spoiling on keeping is reduced considerably.

Desiccated media may be purchased, some of which answer very well.

The Cultivation and Isolation of Bacteria.—The ubiquitous character of bacteria renders it necessary that those under examination should not become contaminated with extraneous organisms. To preclude such contingency, resort is had to certain devices for protection against *pro tempore* aliens. In the absence of draughts, aerial bacteria do not move in a horizontal direction, but merely drop. Consequently, tubes of media are not held with the mouths up during manipulation, but in a more or less horizontal position. Dry cotton-wool is an effectual bacterial filter, and is used for plugging vessels. The part of the plug entering the test-tube is never touched with the hand by conservative workers, who leave an ample portion of plug outside the tube for this purpose. Many, however, regard the large plug as an archaic fetish, and practise economy in cotton-wool by using a loosely fitting plug about an inch long, which all goes in the mouth of the test-tube, and is removed by sterile forceps and held by the top part. It was formerly the custom to always 'flame' a plug before reinserting it, but except when the plug has been dropped, or otherwise exposed to contamination, this ritual is not generally honoured in routine work.

Gelatin Plate Cultures.—Three test-tubes containing nutrient gelatin are placed in warm water at about 40° C. until the contents are liquid. This temperature is

sufficient to keep the gelatin liquid, but not high enough to destroy the vitality of the bacteria which are to be the subjects of experiment. The tubes are then numbered 1, 2, and 3. By means of a platinum needle, which has been previously sterilised at red heat and allowed to cool, after carefully withdrawing the plug, a *mere trace* of the mixture of organisms under examination is introduced into tube 1 and well mixed. If the material is too coherent, attempts must be made to separate the organisms by rubbing them with the point of the platinum wire against the side of the tube below the surface of the gelatin. The plug is replaced and the needle sterilised by passing it through the flame. A platinum loop is sterilised, and when cool, a loopful of the gelatin transferred from tube 1 to tube 2, and the contents well mixed, after which two or three loopfuls from tube 2 are transferred to tube 3. By the above dilution the number of organisms in the third tube is probably so small as to give a satisfactory 'plate.' It may happen that the dilution is carried too far, in which event the plate required is obtained from the second tube. Success in this operation is a matter of experience and judgment. When inoculating tubes the cotton-wool plugs must be held between the fingers, best between the third and fourth, using the back of the hand, and must be carefully returned into the tube after inoculation, without the part that goes in the tube being allowed to come into contact with the surface of the hand or bench. The manner in which plugs and tubes are held is largely a matter of taste, but whatever posture is adopted, the positions of the respective plugs and tubes must be remembered, to avoid mixing them.

The plug of No. 1 tube is flamed and removed, the lips of the tube are flamed, and the contents of the tube poured into a Petri dish which is marked 'No. 1.' The tubes marked 2 and 3 are treated similarly. Petri dishes are from 10 to 20 centimetres in diameter and about 1·5 or 2 centimetres deep, and have loosely fitting covers of the same form as the dishes themselves. The colonies may be examined and counted without removing the lid.

Esmarch's Roll Cultures.—The inoculated liquefied gelatin is distributed in a thin layer upon the walls of a wide test-tube by rotating the tube upon a block of ice

having a horizontal surface, in which a shallow groove has been made by means of a test-tube containing hot water.

Agar Plates.—Tubes containing nutrient agar are placed in a bath of boiling water until the contents are completely melted. The bath is allowed to stand until the temperature falls to nearly 45° C. The tubes are then immediately inoculated and the contents poured into a dish, as previously directed for the preparation of gelatin plates. It is a good plan to warm the dishes or plates to 40° C. before pouring the agar, and, above all, to work quickly, as the agar solidifies at 40° C., and after solidification has begun to take place an even distribution of the medium is no longer possible. Agar plates are usually inverted in the incubator. If left upright, moisture collects in drops on the medium and washes colonies into one another.

Character of Bacterial Colonies.—Gelatin and agar plates (incubated at 22° C. and 37° C. respectively) are examined every day to ascertain the character of the colonies. It occasionally happens that a colony contains more than one species owing to the too close propinquity of the original bacteria, but as a rule each isolated colony is a pure culture. Some bacteria liquefy gelatin more or less quickly, liquefaction being shown by a sinking and the evident local destruction of gel.

For further study of the colonies isolated on plates they may be inoculated into liquid or solid media. Inoculations on to solid media may take the form of a 'stab' or 'streak' culture.

'Streak' Cultures.—Nutrient gelatin or agar is solidified in an oblique position, so as to expose as much surface as possible. The tube is held in a horizontal position to prevent aerial organisms from falling in, and the plug is carefully withdrawn with the third and fourth fingers of the right hand, using the back of the hand. The platinum needle is sterilised by heating to redness in flame, and given time to cool. A trace of the colony is taken up on the point. The needle is carefully passed down the tube so as not to touch the sides, and then gently drawn along the centre of the medium, using a light but even pressure. The needle, on removal, is at once sterilised, and the cotton-wool plug after flaming is returned to the

tube. The whole operation is carried out as quickly as possible, so as to reduce the chance of outside contamination to a minimum.

'Stab' Cultures.—A trace of the growth is picked up on the tip of a platinum needle, which is then thrust into the depth of a tube containing about 10 c.c. of nutrient medium solidified in the upright position, care being taken to introduce the wire in a central line and in a direction parallel with the sides of the tube. The tube may be held and the manipulations carried out in the same manner as described for a 'streak' culture.

'Stab' and 'streak' cultures may be made in the same tube, if this be filled and 'slanted' in such a manner that the slant ends halfway down it.

To show the production of gas, stab cultures in glucose agar, shake cultures in gelatin, or cultures in Durham's fermentation tubes (see p. 37), may be employed.

'Shake' Cultures.—A tube of gelatin is liquefied by heating the tube in a beaker of water at 40° C. The medium is then inoculated with the organism under examination. The plug is replaced, and the contents of the tube gently mixed to distribute the organisms evenly through the medium, care being taken not to allow any of the gelatin to touch the cotton-wool plug. The contents of the tube are allowed to set in cold water. The presence of organisms capable of producing gas in this medium is shown by the formation of bubbles. At the same time, another tube of the medium is inoculated with an organism known to produce gas—as a control. This prevents an error of registering this attribute as negative when really non-production of gas is due to the use of unsuitable medium. The test sometimes fails with gelatin made with meat extract.

Anaërobic Cultures.—Most anaërobes will grow in a deep stab in glucose agar or gelatin. The tube, three-quarters full of medium, is kept in boiling water for five minutes and then cooled. Dissolved oxygen is thus expelled and the medium softened. After the stab is made, the top portion of the medium is gently warmed to seal the top of the needle track.

Air may be excluded from a culture in a fluid medium by running a layer of sterile vaseline or olive oil (about a centimetre thick) on to the medium.

Buchner's Tube.—A large test-tube, having a constriction a little above the bottom, and fitted with a rubber stopper, well vaselined. The inoculated tube is placed in the Buchner's tube, and rests on the constricted portion. Some strong aqueous solution of pyrogallic acid is then run into the outside tube, followed by an equal volume of 20 per cent. caustic potash solution. Without allowing the pyrogallic acid and potash to mix, the rubber stopper is quickly inserted, and the solutions in the tube below the constriction mixed. Stoppered glass bottles may be used for the purpose.

In *Fränkel's method* a large, strong test-tube containing the medium is fitted with a rubber cork, through which pass two glass tubes, bent at right angles just above the stopper, and the ends drawn out. One tube should reach almost to the bottom of the tube, and the other just through the cork. After sterilisation and inoculation, hydrogen is passed through the longer tube and escapes by the shorter one. When all the air has been expelled the tubes are sealed in a Bunsen flame, the shorter tube being done first.

Bulloch's apparatus consists of a large bell-jar with ground flange, which can be luted to a plate of ground-glass with a suitable grease or resin ointment. Through the top, two tubules with ground stoppers, and provided with stopcocks, pass. By means of these hydrogen can be passed through the bell-jar to displace the air, when the stopcocks are closed. A dish of alkaline pyrogallol may be placed at the bottom to absorb oxygen in case of a slight leak.

Discrete colonies of organisms may be obtained by smearing the infected loop over the surfaces of three or four slanted media tubes in turn, without reinfecting. The tubes are incubated in a wide-mouthed stoppered bottle with ample volumes of pyrogallic acid and soda solutions. One gramme of the former should be allowed for every 100 c.c. of air space.

Animal Inoculations.—Animal inoculations may be required, among other reasons, (*a*) to enhance the virulence of an organism which has become attenuated through culture on media—the infective agent may be injected alone, with another pathogenic organism, or with a toxin to lower the animal's resistance; (*b*) to identify an organism; (*c*) to obtain a pure culture.

Rabbits, guinea-pigs, white mice, and white rats, are the animals generally used. The first two may be injected subcutaneously or intraperitoneally. The hair on the abdomen, between the scapulæ, or near the root of the tail, whichever part be chosen for the injection, is clipped, and the skin rubbed with cotton-wool soaked in 1 in 1,000 mercuric chloride solution. For a subcutaneous injection, the skin is pinched up and the needle inserted. The needle is run in for its whole length when a large amount is to be injected. Intraperitoneal injections are made in the lower part of the abdomen; the abdominal walls are pinched up, and the needle passed through the fold and then withdrawn until the point is felt to be free in the abdominal cavity, when the contents of the syringe are emptied. Care must be taken to avoid injuring the intestines. A special curved needle, with the hole about a quarter of its length from the point, is frequently used for intraperitoneal injections.

The large auricular veins of the ear render the rabbit a suitable animal for intravenous injections. The vein selected is rendered prominent by lightly pinching the base of the ear.

For a 'pocket inoculation' a small incision is made in the skin, and, the latter having been separated from the muscles by inserting the point of a pair of scissors and slightly opening them, the tissue is inserted in the cavity. The wounds left after inoculation are closed with collodion, collodion and wool, or with one or two sutures.

Mice are generally inoculated on the back, at the root of the tail. Inoculations may also be made into the anterior chamber of the eye, by rubbing infected material into a scarified surface, or by the introduction of the material in collodion sacs.

Examination of the Dead Animal.—As soon as possible the body is pinned on a board, the dorsal surface down. The forceps, scissors, and scalpels, should be sterilised by boiling in water containing a little sodium carbonate. The animal and board are well soaked in disinfectant solution, and the hair on the abdomen shaved or clipped. The abdomen is seared with a hot iron, and an incision made from the top of the sternum to the pubes; lateral incisions are made, and the skin reflected and pinned out.

A fresh set of instruments is used for the next

incisions, and a third set for removing the organs. The material from which cultures are to be made depends on the organism suspected. The site of inoculation, the spleen, or the blood, may furnish the organism in most abundance. Before any organ is opened it is first seared with the cautery. Blood and peritoneal fluid may be collected in sterile capillary pipettes. Blood is taken from the right ventricle. After dissection the animal is drenched with disinfectant, and, together with the board, is burnt at once. The greatest care must be taken to prevent the dissemination of infectious matter, and in the event of any material being dropped, it must be immediately swabbed up with disinfectant.

Blood Preparations.—Small quantities of blood are obtained by pricking a finger or lobe of the ear with a bayonet-pointed needle after sterilisation of the skin by rubbing with alcohol and ether. For a Widal reaction the blood is taken up in a capillary pipette. For other serum tests Wright's capsules are used. For blood-films, the exuded drop of blood is touched with the edge of a microscopical slide, and then brought in contact with another slide, near its end. When the drop has spread across the slide, the first is gradually drawn or pushed across the horizontal one. The thickness of the film can be varied at will by altering the angle at which the top slide is drawn across the other. For determining the nature of organisms in septicæmia, larger quantities of blood are required—up to 5 or 10 c.c. Such blood is taken, with aseptic precautions, from a superficial vein (the median basilic or median cephalic veins are convenient) with a sterile glass syringe, a tourniquet being applied to produce venous congestion. The blood is introduced in quantities of $\frac{1}{2}$ c.c. into agar plates or broth-tubes.

CHAPTER IV

THE MICROSCOPIC EXAMINATION OF BACTERIA

Living bacteria are observed in 'hanging drops,' and dead bacteria in film preparations or in sections of tissue. For bacteriological purposes, cover-glasses $\frac{3}{4}$ inch in

diameter and of No. 1 thickness are used. Cover-glasses may be round or square, as preferred. They are cleaned with sulphuric acid and potassium bichromate, and kept, when clean, in alcohol, from which they are removed with forceps, and either wiped with a clean rag or passed through the flame to burn away the alcohol. To obtain a satisfactory preparation, the cover-glass must be free from grease. On a clean cover-glass or slide a minute droplet of water or other liquid can be evenly spread, whereas in the presence of grease it will run into pools. A satisfactory film cannot then be expected, and there is a probability of its washing off during manipulation. A cover-glass should never be wiped with a circular or rotary motion, but the wiping rag should be gently drawn across it, taking care, in the case of a cover-glass, that the pressure on both sides of it is equal.

Hanging-Drop Preparation.—Motility is most evident in young cultures, and broth or agar cultures of twenty-four hours old are most suitable. A hollow-ground slide is passed through a Bunsen flame and then laid on a clean bit of filter-paper, excavated side up. A ring of vaseline is painted round the outside margin of the excavation with a match-end. A clean cover-glass is passed through the flame, laid on a clean flat surface, and a droplet of a broth culture placed on the centre (when a culture from a solid medium is used, a droplet of sterile broth or water is placed in the centre of the sterile cover-glass and inoculated with a minute trace of growth). The excavated slide is lifted, turned over, and gently lowered over the cover-glass, so that the drop of culture is in the centre of the well and the ring of vaseline forms a seal. The slide is now turned over so that the cover-glass is uppermost, and placed under the microscope. For organisms of the size of the typhoid bacillus a $\frac{1}{6}$ objective is suitable, but when a higher power is to be used, the edge or centre of the droplet should first be centred with a low-power objective. The light should be diminished, either by nearly closing the diaphragm or by lowering the condenser. Neither the Brownian movement (p. 2) nor movement due to currents in the fluid should be confounded with motility, in which the movement of individual bacteria is independent, progression taking place in different directions. Conversely, motile bacteria,

particularly in cultures more than a day old, have a resting stage, when no motility is visible.

Intra-Vitam Staining.—A drop of a fluid culture is placed on a sterile slide and covered with a sterile cover-glass. A drop of stain is placed in contact with the edge of the cover-glass. On application of a piece of filter-paper to the edge diametrically opposite, the preparation is irrigated with the stain. Non-toxic stains such as neutral red or methylene green are used in 0·5 per cent. aqueous solution. Other reagents may be similarly applied.

Negative Staining.—*Burri's Indian-Ink Method.*—Liquid Indian ink (Chin-chin, Günther and Wagner, etc.) is sterilised either by heating in an autoclave or allowing it to stand for twenty-four hours mixed with one twenty-fifth its volume of tincture of iodine and then centrifuging. A drop of this is placed on a microscope slide, and by the side of it a drop of the culture to be examined. (Dilution of the ink with from one to six times its volume of water is sometimes necessary.) Then the edge of another microscope slide is allowed to rest on the two drops, which mix and run along the line of junction of the slides. The second slide is drawn across the lower one to make a smear as for a blood-film (p. 46). Organisms show up white on a black background.

The Staining of Micro-Organisms.—Bacteria take up the basic anilin dyes with great avidity, and in some cases peculiarity in respect of a certain staining method serves for identification.

Concentrated alcoholic solutions of stains are prepared by allowing a large excess of the dye to digest for some time in strong alcohol, shaking the solution from time to time. The concentrated solutions are then filtered and preserved in stoppered bottles. To increase the staining properties, certain reagents (phenol, anilin, and alkalies) are employed as mordants.

Stains should be filtered before use, otherwise granules of colouring matter may be deposited upon the preparation.

Ehrlich's Anilin-Gentian Violet:

Saturated alcoholic solution of gentian violet	11 c.c.
Saturated aqueous solution of anilin ..	100 c.c.

The anilin solution is prepared by shaking about 5 c.c. of colourless anilin with 100 c.c. of distilled water for some

time, and filtering the solution through a wet filter-paper. Gentian violet can be replaced by 11 c.c. of saturated alcoholic solution of fuchsin or methyl violet.

The prepared stain should not be kept longer than two weeks.

Carbol-Gentian Violet :

Saturated alcoholic solution of gentian violet	10 c.c.
Five per cent. phenol	100 c.c.

Ziehl's Carbol-Fuchsin :

Fuchsin	1 gramme.
Phenol	5 grammes.
Absolute alcohol	10 c.c.
Distilled water	100 c.c.

The fuchsin is dissolved in the alcohol, and the phenol, previously dissolved in the water, is then added.

For ordinary cover-glass preparations this solution is diluted with water in the proportion of 1 : 6.

In staining for tubercle bacilli, it must be remembered that this dye sometimes loses its staining properties with age, and it should be tested on a sputum known to contain the bacillus in large numbers.

Löffler's Methylene Blue :

Saturated alcoholic solution of methylene blue	30 c.c.
Caustic potash solution (1 : 10,000)	100 „

This solution keeps well.

Kühne's Carbol-Methylene Blue :

Methylene blue	1·5 grammes
Absolute alcohol	10 c.c.
Five per cent. aqueous solution of phenol	100 c.c.

Nicolle's Carbol-Thionine Blue :

Saturated solution of thionine blue in 90 per cent. alcohol	10 c.c.
One per cent. aqueous solution of phenol	100 „

Eosin : ½ to 1 per cent. solution in water or alcohol.

Bismarck Brown [(*b*) stain in Neisser's method for diphtheria] :

Bismarck brown	..	..	2 grammes.
Distilled water (boiling)	..	..	1 litre.

Filter.

Pugh's Stain for Klebs-Löffler bacilli, see p. 111.

Leishman (p. 186), Jenner, picrocarmine, and Ehrlich-Biondi triple stains are best bought ready prepared, either in solution or in tablet form. All stains should be kept in the dark when not in use.

Cover-Glass Preparations.—A small droplet of water is placed in the centre of a clean cover-glass by means of a sterile looped platinum wire. The mouth of the culture-tube from which the preparation is to be made is singed in the Bunsen flame. The plug is loosened by a rotary motion, and partially withdrawn. An inoculating needle (straight in the case of a streak culture, looped for a broth culture) is heated to redness, the lower part of the rod being also heated. The needle is held between the right thumb and forefinger, and the plug is withdrawn and held by the ring and little fingers of the right hand. A trace of the growth (preferably from the margin in the case of a streak culture, as the growth is youngest there) is picked up. The needle is withdrawn and the plug replaced. The growth is rubbed up with the drop of water on the cover-glass, and spread over the surface thereof. The needle is sterilised. The cover-glass may be allowed to dry spontaneously, or may be held between the fingers high over the flame. The film is now *fixed* by passing the cover-glass, held in forceps, three times through the Bunsen flame at the same rate as a clock's pendulum swings. This fixing insures the film being thoroughly dry, coagulates albuminous material, causing adhesion of film to the glass, and may tend to diminish the staining capacity of extraneous matter. A drop or two of a filtered stain should now be dropped on the film, or the cover-glass may be floated face downwards on the stain contained in a watch-glass. The stain is allowed to act for from two to ten minutes, according to the preparation used. (The student should ascertain the length of time which gives best results by experiment.) To quicken the staining process, as is necessary in the

case of some organisms, by using hot staining solution, the cover-glass, well covered with the stain, is held by forceps over a low gas-flame until steam just begins to rise from the liquid, when the source of heat is removed. This treatment is repeated at frequent intervals. A better method is to float the cover-glass face downwards upon the staining liquid, which has just previously been heated in a small dish until the steam begins to rise. Great care must be taken not to allow the stain to boil, as this causes a precipitation of colouring matter, which renders the preparation useless. The cover-glass is then well rinsed in running water until no more colouring-matter comes away. The cover-glass is blotted between filter-paper and allowed to dry spontaneously. Some workers make their preparations on the slide instead of a cover-glass.

A small drop of a thick solution of Canada balsam in xylol is placed in the centre of a clean glass microscope slip, and the cover-glass, prepared surface downwards, deposited on the drop of balsam, which then spreads out. The preparation can now be observed by placing a drop of cedar oil on the top of the cover-glass, and examining with the oil-immersion lens. After examination the cedar oil on the cover-glass is carefully absorbed with filter-paper. After a few days the balsam will become hard.

If a permanent preparation is not required, the cover-glass can be examined immediately after washing off the excess of stain by placing on a glass slip, taking care to dry the top surface of the cover-glass before applying the drop of cedar oil, or may be dried and examined in cedar oil or liquid petrolatum.

Smear Preparations.—A cover-glass is brought in contact with the freshly-cut surface of the organ, such as the liver or spleen. Another method is to press the material between two cover-glasses, which are then separated by sliding them apart, leaving a thin layer of material on each. This method is particularly applicable to blood and sputum. The smears are air-dried and stained, as described under cover-glass preparations. (For preparation of blood films see p. 46.) Smears of blood, pus, and organs may be stained by Leishman's or Jenner's methods (see p. 186).

'Impression' Cover-Glass Preparations. ('Contact' Preparations).—A cover-glass is held with a pair of forceps

over a colony (which should not exceed 2 millimetres in diameter), in a slanting position, with one edge resting on the nutrient medium, then allowed to sink gradually down over the colony, and very gently pressed. The cover-glass is carefully lifted with a needle, and allowed to dry spontaneously. The preparation is 'fixed' and stained. This method shows the manner of growth and the arrangement of the organisms.

Staining of Spores.—In ordinary cover-glass preparations spores resist the stain. All unstained spots are not spores: they may arise from faulty staining due to air-bubbles, the use of old staining solutions, or, in the case of old cultures, the organisms may have become degenerate and vacuolated.

Heat Method.—A cover-glass preparation is passed through the flame twelve times in 'fixing,' stained for a few minutes with warm Ehrlich's gentian-violet or Ziehl's fuchsin solution, and then well washed in water. The heating destroys the power of the organisms to take up the stain, leaving only spores stained.

Neisser's Method.—The cover-glass preparation, made in the usual way, is stained with warm carbol-fuchsin solution for about ten to twenty minutes. It is best to float the cover-glass on the surface of the stain contained in a small dish on a sand-bath or piece of asbestos cardboard warmed with the Bunsen. The cover-glass is removed, washed in water, decolorised for a few seconds in a 3 per cent. alcoholic solution of hydrochloric acid, well washed in water, counter-stained with Löffler's methylene blue for three minutes, washed in water, blotted, dried, and mounted. The bacilli will be stained blue and the spores red.

Moeller's Method.—The films are prepared and fixed, and then treated with—(*a*) absolute alcohol, two minutes; (*b*) chloroform, two minutes; washed thoroughly; (*c*) 5 per cent. chromic acid, one minute. They are stained in warm Ziehl's fuchsin for ten minutes, decolorised in 1 per cent. sulphuric acid for a few seconds (this has to be done with care), washed, counter-stained with Löffler's blue for two or three minutes, again washed, dried, and mounted. The spores are stained red and the bacilli blue.

Flagellum Staining.—Flagella possess no affinity for stains unless previously mordanted. The cover-glasses

must be *absolutely clean*, and the growth be well diluted before spreading, to render individuals sufficiently isolated. A trace of an eighteen to twenty-four hour culture of the organism is very gently mixed with a few drops of tap-water in a watch-glass. A minute drop of water is placed on a clean cover-glass which has been well heated over a Bunsen flame and allowed to cool, and a very small loopful of the bacterial emulsion is added. The cover-glass is spread at once, and the material thereon should be sufficiently small to cause the film to dry immediately after spreading. The cover-glass is held in the fingers and passed three times through the flame at such a rate that the heat can be endured by the fingers.

McCrorie's Night Blue Method.—Solution A.—Dissolve 0·5 gramme of McCrorie's night-blue in 20 c.c. of absolute alcohol.

Solution B.—Dissolve 1 gramme of tannic acid in 20 c.c. of hot water. Add 1 gramme of alum dissolved in 20 c.c. of cold water.

Add A to B slowly, shaking gently all the time. Filter.

The stain is allowed to act for two minutes in a warm place.

Muir's Modified Pitfield Method.

A. The Mordant.—

Tannic acid (10 per cent. aq. sol., filtered)	10 c.c.
Sat. aq. sol. mercuric chloride	5 ,,
Sat. aq. sol. alum	5 ,,
Ziehl's carbol-fuchsin	5 ,,

A precipitate forms which may be allowed to deposit or centrifuged.

The clear solution is removed and will keep a week.

B. The Stain.—

Sat. aq. sol. alum	10 c.c.
Sat. alcoholic sol. gentian violet	2 ,,

This keeps two days.

The film is flooded with the mordant and steamed for one minute. After well washing in water for two minutes, it is very cautiously dried, flooded with the stain, steamed for one minute and well washed in water.

Capsule Staining.—By considerably diminishing the light, capsules may be observed in fresh preparations.

But the clear haloes sometimes seen round bacteria in albuminous material must not be confused with capsules. Burri's India ink method and Gram's method often show the capsules very well.

Richard Muir's Later Method.—A thin film after drying is stained with warm carbol-fuchsin for 30 seconds, washed slightly in alcohol and again thoroughly in water. It is placed in the following mordant for a few seconds:

Sat. sol. mercuric chloride	2 parts
Tannic acid (20 per cent.) solution	2 ,,
Sat. sol. potash alum	5 ,,

After well washing in water, the preparation is treated with methylated spirit for about a minute and should then be pale red. After thorough washing in water and counter-staining with methylene blue for 30 seconds, the film may be dehydrated in alcohol, cleared in xylol and mounted, or may be simply dried with filter paper. The bacteria are of a deep crimson, and the capsules blue.

The Treatment and Staining of Sections.—The tissue is first 'hardened,' and then cut into sections with some form of microtome.

Hardening of Tissues.—The most satisfactory hardening reagent is alcohol. It is preferable to pass through increasing strengths of alcohol—*e.g.*, 50 per cent., 75 per cent., and finally absolute—the tissue remaining in each for twenty-four to forty-eight hours. The tissue is cut into pieces 10 to 20 millimetres square and 5 to 10 millimetres thick; these are immersed in the alcohol, which may be changed once. If absolute alcohol alone be used, it may be contained in a wide-mouthed bottle, in the cork of which are fixed several needles. The pieces of tissue are placed on the needles in such a manner that, when the cork is fixed in the mouth of the bottle, the pieces of tissue are just beneath the surface of the alcohol. The alcohol gradually abstracts the water from the tissue, and as that containing the water sinks to the bottom, fresh alcohol constantly comes in contact with the material. Tissues containing much water are naturally more difficult to harden than those containing little.

Before hardening in alcohol, it is usual to 'fix' the tissues, for the better preservation of the tissue element. A saturated solution of corrosive sublimate in water, to

which 1 to 2 per cent. of acetic acid may be added at the time of using, or 10 per cent. formalin, are suitable for the purpose. The pieces of fresh tissue are placed in either solution for twelve to twenty-four hours, washed in running water, and then placed in absolute alcohol, or passed through increasing strengths of alcohol as detailed above.

After hardening, the tissue may be preserved in 70 per cent. alcohol. Müller's fluid (potassium bichromate) and chromic acid are not suitable for hardening tissues for bacteriological work.

After hardening, the tissue is embedded, in order to prepare it for the section-cutting machine.

The Freezing Method.—The tissue can be frozen without preparation beforehand, but where it has been hardened it must be first freed from alcohol. The pieces of tissue are placed in a wide-mouthed bottle, a funnel is stuck in the mouth of the latter, and a stream of water from a tap allowed to run in for one to two hours. The tissues are then soaked in a mucilage of cane-sugar and gum acacia (to which a little carbolic acid or a piece of thymol should be added to prevent the growth of bacteria and moulds) for twenty-four hours, and are then ready for cutting. A piece of tissue is placed on the plate of the freezing-microtome, a little mucilage added, so that it is surrounded with this, and the whole frozen, the freezing being carried out by spraying the under-surface of the plate with ether. This is done by filling the bottle of the apparatus with ether and working the bellows. The sections are cut by working the knife, which should be moistened with water, and the sections as cut are removed with a moistened camel's-hair brush, and immersed in a dish of tepid water for an hour to remove the gum. From time to time the bellows are worked to keep the mass frozen. Compressed carbon dioxide is now often used instead of ether; the jet of escaping gas impinging on the under surface of the plate quickly freezes the section. After the washing with water the sections may be stained, or may be preserved in 70 per cent. alcohol for future examination.

The freezing process is indicated when rapid production is essential. The sections stain readily. It is frequently used during the actual progress of operations. It has

certain disadvantages—the cellular structure may be distorted, the sections are not very thin or regular, and delicate tissues are apt to be torn.

Embedding in Paraffin.—This method gives the thinnest sections and preserves the structures. The material, after hardening, is placed in absolute alcohol for twelve to twenty-four hours, and then in pure xylol until it looks clear. Chloroform may be used instead of xylol. After this it is placed in a bath of melted paraffin wax, in which it remains for six to eighteen hours, according to the size, until thoroughly impregnated with the melted paraffin. The paraffin is kept in the melted state in a special bath or hot-water oven, the temperature of which is regulated by a thermo-regulator: Hearsons make a special form with 'Excelsior' gas regulator. As regards the paraffin wax to be used, opinions differ, but probably one having a fairly high melting-point—*e.g.*, 50° to 52° C. —is the best all round. Some tissues, such as skin, must not remain longer than is absolutely necessary in the melted paraffin, or they become hard and friable, and will not cut. After impregnation the material has to be embedded; a paper mould or small cardboard box (such as a pill-box) is about one-third filled with melted paraffin wax; the prepared pieces of tissue are laid on the centre of the wax layer, then more melted paraffin wax is poured on in such a way as to enclose the material in the centre of a small block of wax. When set, which is preferably hastened by immersion in cold water, the block is trimmed to a suitable form with a knife, and cemented to the carrier of the microtome by softening the base of the mass with a match-flame, and melting the margins with a hot wire. The sections are cut without any moistening fluid.

The sections are next mounted on slides. This is done by placing them in a dish of warm water (a pastry-tin does well, and may be warmed over the Bunsen), the temperature being so adjusted that the paraffin becomes *softened*, but not *melted*, so that the sections become flat. A slide is introduced into the water under a section, which is then lifted up on it, being fixed and adjusted by means of a needle. The water is then tilted off, and the specimen allowed to dry for not less than two to three hours in the warm incubator. If the sections be *thin*, they adhere

sufficiently firmly to the slide to allow staining, etc., without floating off. But if they be thick, or if the staining has to be prolonged, it is preferable to first smear the slide with egg-albumin mixture (egg-white beaten up with a little water, mixed with an equal volume of glycerin, and sodium salicylate, 1 gramme per 100 c.c., added as a preservative, and strained) before picking up the section on it.

Before staining, the slide with section is immersed in xylol (best in a suitable pot or glass cylinder) for two minutes to dissolve out the paraffin, then in absolute alcohol to remove the xylol.

The Staining of Bacteria in Sections.—The following procedure is common to most methods: The sections are rinsed with distilled water to remove alcohol, and then subjected to the action of the stain for a time varying from a few minutes to several hours. The time is in some cases shortened by warming the staining solution. The sections are next washed, preferably in distilled water, and then in some instances decolorised with a suitable reagent; they are again washed, then counterstained if necessary. The sections are now dehydrated with alcohol, and then cleared with xylol, cedar oil, or oil of cloves. Xylol or cedar oil is preferable to oil of cloves as a clearing agent, as it has no solvent action on the stains, and the former does not resinify on exposure to the air, and evaporates without leaving a deposit. Great care must be taken to remove all the water from the section by means of *absolute* alcohol before transferring to the xylol or cedar oil, otherwise the section will not properly clear.* After remaining in the xylol for about two or three minutes, the section, if unattached to the slide, is removed by means of a section-lifter, and then laid out flat by careful manipulation with two small pointed glass rods or needles on a clean glass slide; the excess of clearing agent is removed by careful blotting, with *firm pressure*, with two or more thicknesses of filter-paper. A drop of thick solution of Canada balsam in

* Oil of cloves will clear out of *methylated* spirit, but for xylol or cedar oil *absolute* alcohol must be used. For many purposes methylated spirit may be substituted for absolute alcohol, but the ordinary commercial methylated is unsuitable, as it becomes milky on the addition of water. The *old* methylated spirit (alcohol and wood spirit) must be used.

xylol is dropped on the section, and a cover-glass laid on in such a way that the drop of balsam covers up the section, and extends over the whole under-surface of the cover-glass, as in the case of simple cover-glass preparations. The preparation is now ready for examination with the oil-immersion lens. If the section is fixed to the slide, as is the case with paraffin sections, all the manipulations are carried out on the slide, the slide being flooded with the stains and various reagents, or immersed in pots containing them.

Löffler's Method.—The sections are stained in Löffler's methylene blue for from ten to sixty minutes, then rinsed in distilled water and slightly decolorised by immersing in a 0·5 per cent. solution of acetic acid for a few seconds. The sections are now washed, dehydrated in absolute alcohol, cleared in xylol, transferred to the slip, blotted, and mounted as usual. Carbol-thionine blue may also be used, and often differentiates better than Löffler's blue.

Kühne's Method.—The sections are placed in Kühne's carbol methylene blue for thirty minutes or longer, then washed in water, and very carefully decolorised in very dilute hydrochloric acid (20 drops of strong acid in 100 c.c. water). Thin sections only require to be immersed for two or three seconds. The sections are at once transferred to an alkaline solution (10 drops of a saturated solution of lithium carbonate in 10 c.c. of water), washed in water for a few minutes, dehydrated in absolute alcohol tinted with methylene blue. The sections are now placed in anilin oil, which also contains a little dissolved methylene blue. After being washed in colourless anilin, then in xylol, they are mounted, as usual, in balsam.

Ziehl-Neelsen Method.—Where sputum is to be examined, a little of the material is spread on a microscope slide. If any small yellow caseous particles are present, these should be selected, otherwise the thick portion of the sputum should be used. After spreading, drying, and fixing, filtered carbol-fuchsin solution is dropped on the film, and the slide is heated over a Bunsen flame till steam just rises, care being taken to prevent the stain boiling. The stain is allowed to act for five minutes, fresh stain being added as evaporation takes place. The preparation is now decolorised by dipping alternately

in 25 per cent. sulphuric acid and in water until the film is practically colourless after the last rinse in water. The film is now well washed in water, and counter-stained with Löffler's methylene blue for one minute, again washed in water, and allowed to dry. A drop of cedar oil is added, and the film examined with the oil-immersion objective direct. Any acid-fast organisms present will be stained red.

The method for staining the tubercle or leprosy bacillus in section by this process is as follows: (1) Stain the sections in warm carbol-fuchsin solution for ten minutes. (2) Rinse in water. (3) Decolorise in 25 per cent. sulphuric acid and in water, transferring from one to the other alternately until almost decolorised; a faint pink does no harm. (4) Rinse in water. (5) Counter-stain in Löffler's methylene blue solution for three minutes. (6) Dehydrate in absolute alcohol for half to one minute. (7) Clear in xylol or oil of cloves for five minutes, transfer to slide, blot off excess of clearing agent, and mount with a drop of balsam.

Gram's Method.—Gram's method can be applied equally well to cover-glass preparations and to sections. The process is as follows: (1) Stain the film in Ehrlich's anilin gentian violet or carbol gentian violet for five minutes, or a section for ten to thirty minutes, and drain off the stain. (2) Immerse the preparation without washing in iodine solution (1 gramme of iodine and 2 grammes of potassium iodide dissolved in 300 c.c. of water) for one to two minutes, when the colour changes to a dirty brown. (3) Wash in alcohol until no more colour comes away. (4) Counter-stain in eosin or Bismarck brown. Cover-glass specimens can now be washed, dried, and mounted, while sections require to be further treated as follows: (5) Dehydrate in alcohol. (6) Clear in xylol or oil of cedar, transfer to the slide, if necessary, with the section-lifter, lay out flat, blot off the excess of clearing agent, add a drop of balsam, and mount. (Cover-glass preparations of pure cultures do not require counter-staining. After (3) rinse in water, dry, and mount.) All bacteria do not retain their stain when thus treated. Those which after the alcohol treatment, *i.e.* (3), are still stained are referred to as 'Gram-positive,' while those which are decolorised are known as 'Gram-negative.'

Gram-Günther Method.—The section is treated with a 3 per cent. solution of hydrochloric acid for a few seconds after the first alcoholic washing. By this treatment cleaner and brighter preparations are obtained.

CHAPTER V

THE ACID-FAST ORGANISMS

Most organisms when stained with carbol-fuchsin are readily decolorised by weak solutions of mineral acids. The tubercle, leprosy, and smegma bacilli are, however, notable exceptions, and retain the stain even after treatment with 25 per cent. sulphuric acid or with 30 per cent. nitric acid. This persistent retention of the stain is also shown by some saprophytes (*vide* p. 74), and by some members of the *Streptotricheæ*. It is probable that the degree of resistance of streptothrices to decolorisation is directly proportionate to the vitality of the organism. A pathogenic streptothrix from an old lesion or from one that has been vigorously treated with antiseptics will probably be decolorised, and will be Gram-negative, whereas acid-fast bacteria are Gram-positive. It is believed that some strains of the tubercle bacillus are naturally not acid-fast. In young cultures of tubercle bacilli some, presumably immature, rods are not acid-fast while older ones are.

The Tubercle Bacillus.

Bacillus tuberculosis is a non-motile, slender rod often slightly curved, with rounded ends. It is from 2·5 to 5 μ long and 0·2 μ thick (see also p. 159). When stained it often shows unstained areas ('beading') which a minority of observers consider to be spores. In the moist state the thermal death-point is 60° to 65° C. Milk which has been kept at 70° C. for thirty minutes (Pasteurisation) is regarded as safe, but there is some question as to whether tubercle bacilli are not protected by the pellicle that forms on milk heated in an open vessel. The Board of Agriculture recommend an exposure to 85° C. for fifteen minutes for rendering creamery products harmless.

Culture.—The organism is aërobic and facultatively anaërobic. On ordinary media growth is either absent or scanty. It grows best at blood-heat, growth being extremely slow; no appreciable evidence being shown under four to six weeks. Primary cultures are made on Dorset's egg medium, glycerin agar *cum* egg-yolk, or glycerinated potato. In glycerin broth it develops as a floating pellicle. On glycerin agar it gives a cream or brownish-yellow film which is dry and wrinkled. A pure culture can be obtained by the inoculation of tubercular sputum into a guinea-pig. After three to six weeks the animal is killed, and matter from the tubercles is streaked over glycerin potato (a semicylinder of potato in a Roux's potato tube, the bulb being filled with 5 per cent. glycerin). In six or eight weeks a fair number of the tubes will show a growth. The organism does not liquefy gelatin. On repeated subculture it becomes longer and thicker; clubbed and branching forms may develop, which leads some to class the bacillus with the trichomycetes. As a saprophytic habit is developed the virulence is diminished, but can be restored by passage through an animal.

Staining Reactions.—Ordinary stains have little effect on the organism, but it stains well by Gram's method and the Ziehl-Neelsen method. Much concluded that three forms of the organism exist: (1) The ordinary 'acid-fast' bacillus; (2) a bacillus that is not acid-fast; and (3) free granules also not acid-fast. Whether these last two forms are degenerate organisms or resistant types remains unproved. At any rate it appears unquestioned that the non-acid-fast granules on injection into animals produce tuberculosis due to the ordinary acid-fast bacillus.

In addition to being acid-fast, the tubercle bacillus is also "alcohol-fast," a property that serves to distinguish it from the smegma and similar bacilli which lose their stain in alcohol treatment. Inasmuch as smegma bacilli may occur in urine, sputum, and other material frequently examined for tubercle, it is advisable to apply alcohol to fuchsin-stained preparations as a routine process, especially as it can be combined with acid treatment. Instead of using 25 per cent. sulphuric acid the fuchsin preparation is decolorised in acid alcohol (3 per cent. hydrochloric acid in alcohol) till almost white.

Channels of Infection.—The sputa of consumptives are almost certainly responsible for most infections. The tubercle bacillus is, for a non-sporing organism, tenacious of life, and when sputum is allowed to dry, especially when protected from light and air, may easily be disseminated in dust. In the acts of coughing, sneezing, and talking, minute droplets of fluid are disseminated. Flügge has shown that these droplets may remain in the air for some time, and also that, when from consumptives, they often contain tubercle bacilli. The inhalation of either tubercle-laden dust or droplets of saliva from consumptives must be regarded as a standing menace. Infection may take place through the alimentary tract, especially in infants, by the ingestion of tuberculous milk or meat. Flies can carry the disease. A phthisical patient may swallow his own sputum, and thus infect his alimentary tract. Von Behring considers that tubercle bacilli taken in milk during infancy may remain dormant for years, and then set up infection. Accidental inoculations through the skin with tuberculous material, principally the result of post-mortem examinations (' pathologists' warts '), are seldom serious. Lupus is presumably acquired by inoculation through a wound or abrasion of the skin.

Hamburger and Schlossmann practically agree that 90 per cent. of all children up to the completed twelfth year are infected.

Autopsy statistics show tuberculous lesions in 58 per cent. of adults according to Beitzke. Other statisticians' figures run up to 90 per cent. and more.

Pathogenesis.—Scarcely any portion of the human frame is immune to tubercle. Whooping-cough, pneumonia, typhoid, measles, influenza, diabetes, and syphilis, predispose to the disease, as do also intemperance, poverty, uncleanliness, overcrowding, and lack of ventilation. Trades in which operatives are exposed to dust of an irritating kind, which are carried on in overcrowded, hot, or badly-ventilated rooms, in which workers adopt a cramped posture, or in which exposure to quick alternations of temperature is frequent, show a heavy mortality from the disease.

The disease is not hereditary in the true sense of the word. An infected child of a tuberculous mother is generally the subject of postnatal infection. Nevertheless,

intra-uterine or placental infection does occasionally occur. Although human semen may contain tubercle bacilli, Jordan considers that transmission by the male parent is very unlikely to occur, and there is only one recorded case of an infected ovum. A 'tendency' to the disease may be inherited, but it is more logical to attribute the occurrence of the disease in a family to the intimacy of family relations allowing abundant opportunity for infection.

The lesions produced in tuberculosis have more or less similarity to those of leprosy. The little yellowish nodules or tubercles (to which anatomists first applied the term 'tuberculosis') are non-vascular, and vary in size from that of a pin's head up. They consist of vast aggregates of cells (hence the term 'Granulomata'). In the centre of a young tubercle a mass or masses of protoplasm are found ('giant cells'). (Giant cells are much less common in leprosy.) In the giant cell is a ring of tubercle bacilli, and around this zone and arranged round the periphery is a ring of nuclei. Around the giant cells epithelioid cells with large nuclei are found, and around these a collection of lymphoid cells.

Although tuberculosis is best known as a pulmonary disease, the glands, skin, bones, peritoneum, urinary organs, meninges, etc., are also frequently attacked. Widely diverse findings are recorded of the frequency with which the tubercle bacillus occurs or is found in the blood. Minchin assumes tubercle bacilli in the host to be suspended in 'shut-away fluid' which protects them from the influence of germicides and sera.

Bovine Tuberculosis.—Cattle, particularly those kept in insanitary sheds, are very liable to tuberculosis. The bovine organism may reach others than those in contact with the animal, through the medium of the milk or flesh. When the udder is affected, the bacilli will probably be found in the milk, but even when the udder is free from tuberculosis, the bacilli may find their way into the milk, from the fæces, uterine secretion, or sputum, according to the locality of the infection, unless precautions are taken during milking. The bovine bacillus is shorter, thicker, and less readily cultivated than the human one. These considerations, with others, led Koch in 1901 to question the communicability of the bovine tubercle to man. A Royal Commission was appointed in

the same year, and they arranged human tubercle bacilli in two classes—those growing with difficulty on certain artificial media, which they called *dysgonic*, and those growing readily on the same media, which they called *eugonic*. Experiments with dysgonic bacilli of human origin gave results identical with those made with bovine bacilli. In a few cases both the human and bovine types may be found together. As a rule, pulmonary lesions in man are due to the human type of bacillus, but sometimes the bovine tubercle bacillus is solely responsible. (Kossel found two cases in 709 cases of phthisis.) See p. 265.

The majority of cases in which the bovine tubercle bacillus produces lesions in man are cases of alimentary tuberculosis: cervical gland and primary abdominal tuberculosis. In the latter class of cases at least the tubercle bacillus has unquestionably been swallowed. The Commissioners stated their conviction that a certain number of cases occurring in the human subject, especially in children, are caused through the introduction into the body of bovine bacilli, the majority of cases being caused through tuberculous milk.

As long ago as 1886,* Harries stated from clinical observations that lupus was not due to tubercle bacilli as then known to infect human beings, and a few years ago the opinion that lupus was an infection with the bovine type of tubercle bacillus gained credence. Nine out of the twenty cases of lupus investigated by the Commission proved on culture to be bovine tubercle bacilli, though generally they were of feeble virulence. Park and Krumwiede found that from 6 to 10 per cent. of all deaths due to so-called surgical tuberculosis were caused by bovine tubercle bacilli, the cases of tuberculous adenitis and abdominal tuberculosis of children being more often caused by the bovine than by the human type. The same was found true, though to a less extent, in adults. Stiles says that 90 per cent. of surgical tuberculosis seen in Edinburgh was due to bovine tuberculosis and 90 per cent. of the gland cases were due to infection through the tonsils by milk. Some cases of tuberculous joints which show no improvement under human tuberculin treatment rapidly undergo cure with bovine tuberculin.

* 'Lectures on Lupus': A. Harries, M.D., and C. Campbell, M.D. Baillière, Tindall and Cox. 1886.

The Royal Commission did not conclude that the human and the bovine types represent two distinct organisms, but preferred to regard these two types as varieties of the same bacillus, and the lesions which they produce, whether in man or in other mammals, as manifestations of the same disease. Dr. Arthur Eastwood, the bacteriologist to the Commission, after mention of the remarkably stable characters of the viruses, says: 'I find that underlying all the mammalian viruses which I have investigated there is an essential unity of characteristics, the differences observed being differences of degree but not of kind. On artificial culture media they all grow in the same way, though they differ quantitatively in the amount of growth yielded. In the tissues of suitable experimental animals they all produce lesions histologically characteristic of mammalian tuberculosis, though they differ in the intensity of the tissue changes which they set up under similar experimental conditions.' As the Commission have conclusively shown that many cases of fatal tuberculosis in the human subject have been produced by the bacillus known to cause the disease in cattle, they are emphatic that the possibility of such infection cannot be denied. Of fifty-nine cases of tuberculosis in pigs investigated by the Commission fifty yielded the bovine virus, three the human, five the avian, and one a mixture of the bovine and avian.

Butter, skimmed milk, and butter-milk will contain tubercle bacilli if made from tuberculous milk. The use of the mixed milk of a herd, although perhaps reducing the risk, does not entirely remove it. Desirable though it would be to prohibit the use of meat from animals suffering from any manifestation of tuberculosis, economic considerations have to be regarded. It is found that the fat and muscular tissues are seldom involved, the lungs, liver, and pleura ('grapes') being the organs most often diseased. The Royal Commission on Tuberculosis (1898) recognised the fact that tubercle bacilli are seldom encountered away from diseased areas, and recommended that, provided the carcasses be otherwise healthy, in the restriction of lesions to the lungs, thoracic lymphatic glands, liver or pharyngeal lymphatic glands, the non-tubercular portions of the carcass should not be condemned.

In the German *Freibanks*, after removal of tubercular areas, the meat is sterilised, and then stamped and sold as inferior. It has been shown that the tubercle bacillus is not destroyed, if in the centre of a joint of meat over 6 pounds in weight, by the ordinary method of cooking.

The Local Government Board has ordered that 'stripping' (removal of 'skin' from inside of the ribs or flanks, with a view to effacing signs of the disease), in the case of foreign dead meat, shall be sufficient reason for condemnation. It must not be overlooked that, after removing tubercular organs, a butcher may use the same knife for cutting up a carcass, and thus infect healthy meat.

Tuberculosis is rare in new-born calves, and, if removed from a tubercular mother at birth and properly treated, they will not, as a rule, develop the disease. In *Bang's method* for eliminating tuberculosis, the herd is tested with tuberculin, those that react or which are suspicious being isolated. The herd is divided into two sections, which are separated from one another, and have separate attendants and separate buildings; they are, however, allowed to mix when out in the fields. Every six months the healthy side is tested with tuberculin, and any beasts that are found to react are placed on the infected side, while all calves are placed on the healthy side. The animals on the tuberculous side which are obviously tuberculous are got rid of, but those that are apparently healthy are used for breeding. *Ostertag's method* consists in the elimination of cows suffering from 'open' tuberculosis (*i.e.*, where there are open tuberculous lesions in organs with means of external communication, such as in the udder, lungs, intestines, and urino-genital organs) from the others.

Avian Tuberculosis.—Fowls, pheasants, turkeys, and pigeons suffer from the disease, which attains an extraordinary virulence in insanitary fowl-houses. Ducks and geese are immune. Whereas growth of the human bacillus ceases at 41° C., the avian organism grows well at 43° C. Its cultures on solid media are softer, more greasy, and less tightly packed than those of human tuberculosis. The rabbit is readily infected with it, mice, horses and swine are susceptible, while the guinea-pig shows much

greater resistance. Inasmuch as bacilli with typical avian characteristics have been obtained from human cases, it seems highly probable that avian tuberculosis may be a source of infection.

Loewenstein found that the ovary was always diseased in affected fowls, and the bacilli were also present in the yolk and in the membranes of the ovum. He found that even boiling eggs to hardness did not destroy the vitality of the bacilli and thought human beings were infected through eating raw or imperfectly cooked eggs. On the other hand, the Commission, comparing the mammalian with the avian viruses, found differences not merely of degree but of kind, though sometimes lesions of a chronic type are produced that are indistinguishable from infections set up by mammalian bacilli. In their final Report they dismissed the infection of man from tuberculosis in birds as a negligible factor.

Tuberculin.—*Koch's Old Tuberculin* is prepared by growing tubercle bacilli in glycerin veal broth. A copious film formation being needed, flat flasks are used, so that a comparatively large surface is exposed to the air. After six to twelve weeks the culture is evaporated to one-tenth its bulk on a water-bath, and then filtered through a Pasteur filter.

The injection of 0·002 c.c. into a tuberculous person gives rise to laboured breathing, malaise, and pyrexia, great inflammatory reaction and necrosis occurring round the tubercular focus. If injected into a patient in whom phthisis is dormant, it is very apt to cause the old trouble to break out afresh.

In the diagnosis of tuberculosis in cattle it is very valuable, the failures being only about 2 per cent. The injection of 0·1 to 0·2 c.c. causes a rise of temperature of 2° to 3° F. above the normal in from eight to twelve hours.

Koch's New Tuberculin is prepared by drying and pounding young and virulent tubercle bacilli, and extracting with water; the emulsion is then centrifuged. The supernatant fluid (TO) is now discarded. The residue left in the centrifuge is dried, triturated and centrifuged as before, these processes being repeated until hardly any solid residue is left. The whitish, opalescent liquids resulting from these operations are mixed, and constitute TR.

TR possesses immunising power with but little reaction. The fluid TR is made to contain 2 milligrammes of solid matter in the cubic centimetre. This is diluted with sterile salt solution to the required strength. If reaction occurs, this must be further diminished. Injections are made every other day with slightly increasing doses, so that there is never a rise of temperature of over 1° F.

Koch's Bazillenemulsion is a suspension of pulverised bacilli in equal parts of water and glycerin.

The reaction of a tuberculous individual to tuberculin is due to anaphylaxis. It differs from most anaphylactic reactions as it is accompanied by rise and not fall of temperature. A dose of tuberculin, however, does not sensitise the animal to a second injection, this preparation stage taking place in the tuberculous tissues.

The sera of animals immunised in various ways have been employed in treatment, but only with occasional success.

Notification.—Tuberculosis in man is compulsorily notifiable throughout England and Wales. Tuberculosis with emaciation in any bovine animal and tuberculosis of the udder in cows are notifiable under the Tuberculosis Order of 1913.

Segregation (Newsholme's Law).—International statistics show reason for believing that the reduced mortality from tuberculosis is largely due to the increasing extent to which advanced cases are treated in general institutions, and thus segregated from their families, instead of receiving outdoor relief and thus infecting them.

The term '**pseudo-tuberculosis**' has been applied to a number of conditions the common feature of which is the presence of tubercle-like nodules in the tissues. Such may be caused by pathogenic *streptotricheæ*, yeasts and moulds, parasitic worms, etc. See also *B. pseudo-tuberculosis* (p. 125), and Johne's bacillus (p. 70).

Laboratory Diagnosis.—*Sputum.*—The Ziehl-Neelsen method is used (p. 58). Although tubercle bacilli are resistant to putrefactive changes, a little 5 per cent. phenol should be added when the examination is delayed. Where it is advisable to concentrate the specimen's content of tubercle bacilli, one part of 'antiformin' (a mixture of equal parts of Liquor sodæ chlorinatæ [B.P.] and 15 per cent. caustic soda solution) may be added to

from four to six parts of sputum according to the consistence of the latter. The resulting mixture is shaken well and allowed to stand for two or three hours till solution occurs, which may be hastened by placing the mixture in the incubator. On centrifuging, the tubercle bacilli are obtained concentrated in the sediment, which is generally very slight. Other acid-fast organisms are occasionally met with (p. 74) in sputum. Negative evidence is only of value when repeated examinations have been made.

Milk.—See p. 222.

Urine.—To exclude the smegma bacillus (pp. 61, 73), the urine may be drawn off with a catheter. The urine should be allowed to stand in a conical glass for twenty-four hours, or should be centrifuged. (Russ's electrical method (p. 10) often reveals the presence of tubercle bacilli when centrifugal methods fail to do so.) Should a catheter not be used, the meatus urinarius and the labia (or glans penis) should be cleansed by sponging. The first portion of urine should be discarded. The film is stained as for sputum, but after the acid treatment is put in absolute alcohol for one minute (or, after staining, is put in alcohol containing 3 per cent. hydrochloric acid for ten minutes—Housell's method) to decolorise any smegma bacilli, washed in water and counter-stained. Inoculation of guinea-pigs may also be used.

Agglutination Reaction.—The serum of tuberculous cases agglutinates the tubercle bacillus, but the technique is difficult. Special cultures of the tubercle bacillus have to be employed, or dried and triturated cultures may be used (Koch).

Tuberculin Reaction.—See p. 67.

Von Pirquet's Cutaneous Reaction consists in the application of tuberculin to a scarified surface. In twenty-four hours red papules arise. It gives positive results in healed cases of adults as well as in those where the disease is active. It has more significance with children under three years. Lapage says the method gives a lower percentage of results than the subcutaneous, and that a single negative result is not of much value. Lapage's statistics on this method show that at the end of the school age between 50 and 60 per cent. of children have become infected with tuberculosis.

Moro's Ointment.—An ointment of tuberculin in lanolin. The reaction is similar to that of von Pirquet.

Calmette's Ophthalmo-Tuberculin Reaction.—A solution of old tuberculin freed from glycerin by precipitating with alcohol is instilled into the conjunctival sac. In a tuberculous subject a congestion of the conjunctiva takes place. Calmette does not consider it of any value in prognosis, and it is suggested by some as not safe to use.

The Opsonic Index.—A healthy person's blood is centrifuged in normal saline solution containing sodium citrate. The leucocytes deposited are centrifuged with normal saline. An emulsion of moist dead tubercle bacilli (such as can be purchased) in 1·6 per cent. salt solution is prepared. Equal volumes of washed leucocytes, bacterial emulsion, and the patient's serum are drawn up into a capillary pipette, and then blown out, mixed, and drawn up into the pipette. The tip of the pipette is sealed in a flame, but the blood must not be heated, as this destroys the opsonic power, and an identical mixture is made, using normal serum. The pipettes are incubated at blood-heat for fifteen minutes, and blood films are then prepared from each and stained. The number of tubercle bacilli in at least fifty polymorphonuclear cells is counted. The index is found by dividing the number of organisms in the specimen by the number in the control. Experience and care are necessary to obtain comparable results. It is general to examine the blood before and after some disturbance of the focus, by walking and breathing exercises in the case of pulmonary affections, or the application of a Bier's bandage or massage if the focus be localised and approachable. If fever be present, an inverse relationship of opsonic curve to temperature suggests tubercular trouble.

Johne's Bacillus.

During life, Johne's disease, a complaint peculiar to the ox and sheep, is sometimes mistaken for tuberculosis. The chief symptoms are diarrhœa and wasting. The walls of the diseased bowel contain large numbers of an acid-fast organism, morphologically indistinguishable from the tubercle bacillus. Twort says that in the first generation the organism grows long, with branching and club

formation. In subcultures it is smaller, being in the second or third generation about the same size as the tubercle bacillus. It will not grow on ordinary media, but Twort not only devised a medium (of glycerinated eggs mixed with dried, dead, acid-fast bacilli) but also prepared an effective diagnostic vaccine from the cultures. Twort and Ingram incline to the view that the causative organism of Johne's disease in sheep is the same as that of cattle. Besides the animals mentioned only the goat seems affected by injections of the organism. Infected cattle are said to react to avian tuberculin.

The Leprosy Bacillus.

Morphology.—The *B. leprœ* is a long slender rod, usually straight, with more or less pointed extremities. So far as is known, it is non-motile, and produces no spores. It is Gram-positive and acid-fast, although it stains more rapidly and decolorises more quickly than the tubercle bacillus. Large numbers of the bacilli are found, but a large proportion, especially in the older lesions, are dead. By Ziehl's method, using 20 per cent. nitric acid, and counter-staining with polychrome blue, young bacilli are stained red, older bacilli violet, and granular bacilli blue.

Kedrowsky found a diphtheroid leprosy organism which was not acid-fast. Bayon, the Research Bacteriologist at Robben Island, thinks it to be a stage in the development of the typical bacillus. A disease similar to leprosy may be found in rats in most parts of the globe. Sometimes this runs to a glandular type, sometimes to a skin affection; acid-fast bacilli indistinguishable from typical leprosy bacilli are present in enormous numbers, and Dean has found an organism similar to Kedrowsky's bacillus in cultures.

Culture.—Peptone glycerin serum, human blood-serum, fish broth, placental juice glycerin agar (Bayon), symbiotic culture with amœbæ (Clegg), and eggs have been stated to allow the growth of the bacillus.

Inoculation.—Several workers have reported successful attempts to inoculate man, monkeys, and white mice, but the results are much criticised. Bayon, however, says that Kedrowsky's diptheroid produces leprous lesions in the rabbit, rat, and mouse, and that the resulting lesions are similar to those caused by certain strains

of human tubercle. Couret says that multiplication of the leprosy bacillus occurs in inoculated fish, frogs, turtles, and other cold-blooded animals.

Pathogenesis.—Two types of leprosy are recognised: the nodular (or tubercular), in which the new formation has its seat in the skin or mucous membrane; and the anæsthetic (lepra anæsthetica), in which the nerves are chiefly affected. In the skin variety the hands and face are mostly affected, and larger or smaller swellings appear (red or blue in colour), which become hard. These tubercles consist of granulation tissue, and may ulcerate and cicatrise, producing great deformities. In the anæsthetic form the nerve trunks become the seat of the granulations in the interstitial connective tissue. The spindle-shaped swellings compress and separate the nerve fibres. Besides the anæsthesia, other evidences of interference with nerves, such as eruptions, alterations in pigmentation, and ulceration, frequently occur.

The leprosy bacillus has been found in most of the tissues and viscera, though it occurs more in the liver and spleen than in the kidneys and brain. It has been detected in the blood during febrile paroxysms in the later stages. Enormous numbers are found in cutaneous and other nodules, and in the discharges therefrom. Stitt says the earliest lesion is probably a nasal ulcer at the junction of the bony and cartilaginous septum. Scrapings from this ulcer may give an early diagnosis.

Lepers frequently react to Old Tuberculin and give positive Wassermann tests. The significance of these findings is uncertain, but it has been suggested that nothing more than coexistence of tuberculosis or syphilis respectively is indicated. Fletcher (*Jour. Hygiene*, July, 1915) says that lepers give negative luetin reactions and thinks the positive Wassermann due to a cause other than syphilis. According to Stanziale and Serra the Wassermann reaction is positive in rabbits which have been successfully inoculated with Kedrowsky's diphtheroid.

Channels of Infection.—The use of fish, especially in the putrid condition, was considered by Hutchinson to be a causative factor, but the disease occurs among the Basutos, who never eat fish, and among the vegetarian Brahmins. David Walsh modifies the idea by pointing out that dried fish, a common article of diet amongst

Eastern nations, may be contaminated by the nasal discharges of lepers, the fish being merely the passive agent whereby transmission is effected. Deficiency of salt has been suggested as conducive to the disease. Insects—*e.g.*, flies, fleas, lice, the itch parasite, etc.—may perhaps disseminate the disease, and Goodhue claims to have found the bacilli in the gnat and bed-bug. MacLeod thinks the most common mode of invasion is via the nasal mucosa and upper respiratory tract. The bacilli may gain an entrance through the mouth and infect the tonsils, and they have been found in the sputum; the genital organs and the skin may also allow invasion. The remarks made about the inheritance of tuberculosis seem to apply to leprosy. Although contagious, the extent to which it is propagated by this means is said to be exceedingly small.

The resolutions of the British and Colonial delegates to the International Conference on Leprosy at Bergen (1909) included *inter alia* the following: Leprosy is spread by direct and indirect contagion from persons suffering from the disease. Leprosy is most prevalent under conditions of personal and domestic uncleanliness and overcrowding, especially where there is close and protracted association between the leprous and non-leprous. In leprosy an interval of years may elapse between infection and the first recognised appearance of disease. It is a disease of long duration, though some of its symptoms may be quiescent for a considerable period and then recur. The danger of infection from leprous persons is greater when there is discharge from mucous membranes or from ulcerated surfaces.

The Smegma Bacillus.

The smegma bacillus closely resembles the tubercle bacillus in size and shape, and also in being acid-fast and Gram-positive. It is found in the preputial secretion and between the labial folds of the vulva. It occurs on the skin, and also, it is stated, in the ear, on the tongue and teeth, in the sebaceous secretion, and perhaps in the sputum. It exhibits a marked preference for those secretions containing fatty matters. As found on the bodies of lower animals, variations in appearance were

described by Cowie, and the name is now generally held to include a number of allied organisms. Inoculation of animals gives negative results. Czaplewski found it to grow, though with difficulty, on serum, glycerin agar, and in broth. Subcultures grow more freely. Muir and Ritchie first showed that after staining and decolorising with acid in the Ziehl-Neelsen method, a minute's exposure to alcohol sufficed to remove the red stain from smegma bacilli, and thus to prevent their being mistaken for tubercle bacilli of which there is sometimes a risk, especially in the examination of urine. In practice, Housell's combination of alcohol with the acid is most frequently used (p. 61).

Pappenheim's solution* has been recommended for decolorising acid-fast bacilli other than tubercle after fuchsin staining and washing, but it appears to have little, if any, advantage over acid alcohol.

Other Acid-Fast Organisms.

A number of acid-fast bacilli have been found in milk, butter, manure, and grass. Most of them grow freely on ordinary media at room temperature, often producing a pigment, yellow or brown. The best known among them are the Timothy-grass bacillus (p. 159) and the 'Mist-bacillus' of manure. Some are pathogenic to guinea-pigs, and the lesions produced may simulate those of tuberculosis to some extent.

Petri and Rabinowitch's 'butter bacillus' is found in butter fairly frequently, and when intraperitoneally injected with butter into guinea-pigs produces nodules that may be confused with tubercles.

Acid-fast organisms have been found in sputum when tuberculosis could be negatived on clinical and other grounds. A hint as to their true character may be sometimes obtained from the very small number seen on prolonged search. Doubt as to identity should also be expressed when the shape and size are not those usually found in the case of the tubercle bacillus. Acid-fast bacilli have been described in bronchitis and pulmonary

* *Pappenheim's solution*, 1 part of corallin (rosolic acid) in 100 parts of absolute alcohol, to which methylene blue is added to saturation; 20 parts of glycerin are then added.

gangrene. Wyatt Wingrave (*Medical Press*, 1914, 32) says simple acid-fast bacilli are found in chronic ear discharges, atrophic rhinitis, fæces, lingual accumulations, and in fact in any situation where bacilli grow in the presence of fat. Sanguinetti has found them in distilled water.

CHAPTER VI

SPORE-FORMING PATHOGENIC ORGANISMS

Of the many organisms recognised as distinctly pathogenic, only six are known to produce spores—viz., *B. anthracis*, *B. tetani*, *B. botulinus*, the bacillus of malignant œdema, and two organisms pathogenic for animals alone—*B. Welchii* and *B.* (*Clostridium*) *Chauvœi*. With the exception of *B. anthracis*, they are anaërobes.

The Anthrax Bacillus.

Morphology.—*B. anthracis* varies in length from 5 to 6 μ, in breadth from 1 to 1·5 μ. It is aërobic, and facultatively anaërobic. It is not motile, is usually straight, and has square ends, which are characteristic. In the blood, where it occurs singly or in short chains, the ends of the bacillus are slightly convex; on cultivation they become slightly concave, but neither modification dispels the characteristic squareness of appearance. Round and oval involution forms are often seen in old and attenuated cultures. In the fresh state the threads do not show segmentation; this is seen only on staining.

In cultures chains of great length are formed. When the bacilli have a supply of oxygen, and the temperature is between 20° and 38° C., ellipsoidal central spores are developed. Spores are not found during life, being only developed after death, when the bloody discharges come in contact with air. A variety, first obtained by Behring, which is sporeless for many generations, is produced by culture at 42° C., or on nutrient gelatin containing 0·1 per cent. of phenol.

Culture.—On a gelatin plate small spherical colonies develop in the depth, which consist of closely twisted bands of bacillar chains. When the growth reaches the

surface, spikelets at once begin to radiate over this in wavy convolutions, liquefying the gelatin. This stage is usually reached in two days, and is most characteristic. In the gelatin stab, growth takes place along the needle track, fine branching filaments often growing out into the gelatin ('inverted fir-tree growth'). Liquefaction commences at the top of the stab, proceeding downwards in a horizontal plane, upon which a mass of bacilli rest, leaving the gelatin above clear and liquid. In broth a flocculent growth forms at the bottom of the tube, the bulk of the broth remaining clear and no pellicle forming. On agar a thick, grey-white, sticky growth takes place, and on potato a considerable white growth, both usually containing a large number of spores. Blood-serum is slowly liquefied. An alkaline reaction is generally favourable to the growth of this organism, although it grows well on potatoes, which are normally acid. The bacilli stain well by Gram's method.

Pathogenesis.—In the human subject the disease appears as—(1) *malignant pustule*, a localised swelling caused by direct inoculation, and (2) *woolsorter's disease*, a general infection caused through inhalation of spores during the handling of wools, hides, and fleeces. Infection may also take place through the digestive organs (*intestinal anthrax*). A single case of the lesion appearing in the larynx is reported. In cattle the disease is known as 'Siberian plague' or 'splenic fever,' the latter cognomen being due to the much swollen and soft condition of the spleen found in infected animals. In man, enlargement of the spleen is less marked, and the disease appears to take the form of a toxæmia.

It is pathogenic to the following animals, which are arranged roughly in order of their susceptibility: mice, guinea-pigs, rabbits, sheep, cattle, horses, man, goats, and pigs. Algerian sheep, dogs, frogs,* and white rats are immune.

In susceptible rodents there may be considerable inflammation around the seat of inoculation, and the subcutaneous connective tissue may be distended with a bloody gelatinous exudation. If the tissue is examined microscopically, the blood is found to be full of bacilli,

* Unless the frog is warmed to 37° C.

which in some places may have so distended the capillaries as to have ruptured them and escaped into the surrounding tissue. Anthrax, once introduced, may become endemic in a field in the following manner: The infected animal dies; the bacilli in the bloody discharges that come in contact with the air develop spores, which may be blown about on to the surrounding soil, and remain dormant there for long periods. Animals feeding on grass growing about this spot would be liable to infection. The bacilli, if developed, might be killed by the gastric juice, but the spores could withstand its action and enter the circulation.

Pasteur suggested that after the burial of an animal, worms might bring the spores to the surface. Klein found that all bacilli and spores are killed within a week by putrefaction if the body be left intact. Outbreaks among horses and cattle have occurred through infected oats and linseed cake. The bacillus has been found in effluents, and cattle drinking therefrom have been infected. In one case, foreign bone manure conveyed the bacillus to a workman. The chief danger to operatives in industries involving the handling of wool, hides, and horsehair appears to lie in the small clots of blood on the same. The Bradford Anthrax Investigation Board urge that all blood-stained material should be thrown out before any process of combing takes place.

A highly satisfactory immunity, which, however, is transitory, seldom lasting more than a few months, may be conferred upon susceptible animals by injection of a culture attenuated by growth at 42·5° C. for fifteen days, followed twelve days later by a second culture attenuated by ten days' growth at the same temperature (Pasteur's vaccine).

Cases of anthrax in animals are dealt with under the Anthrax Order of 1899 issued by the Board of Agriculture.

Serum Treatment.—Marchoux vaccinated sheep with an attenuated culture, and then injected them with increasing doses of virulent cultures. Sclavo employs the ass, immunising by the same method, and the antiserum so prepared has been used with success in the treatment of anthrax in man. The serum is bactericidal rather than antitoxic.

Disinfection.—No danger is apprehended from the handling of wools from Australasia and the Argentine, but precautions are necessary when dealing with horsehair from China, Siberia, and Russia, and with Persian wool. While the vegetative forms are quickly killed by heat and disinfectants, 5 per cent. phenol acting for at least twenty-four hours is required for the destruction of the spores, and the disinfection of hides, hair, and wool is therefore difficult. For hides and skins the use of formaldehyde is impracticable, since it so injures the consignments as to prevent them being turned into good leather. Legge thinks it doubtful if there is any way in which hides to be afterwards tanned can be effectively disinfected.

Constant Ponder concludes the best method for destroying the infection on hides to be that devised by Seymour Jones. The process involves treating the hides for twenty-four hours in a soak containing 1 per cent. of formic acid and 0·02 per cent. mercuric chloride, and then transferring them to the ordinary brine pit. This does not interfere with the subsequent processes involved in transformation into leather. Hewlett found the process to be satisfactory provided the strength of the solution was doubled or trebled when dealing with horsehair. Forty-eight hours' exposure to a solution containing 2 per cent. hydrochloric acid and 10 per cent. sodium chloride (Schattenfroh method) is also satisfactory (*Lancet*, August 7, 1915).

Webb and Duncan's experiments seem to show that, leaving out of consideration white or grey hair, which is liable to change colour, no injurious effect is produced on horsehair by steam disinfection, provided the temperature does not exceed 218° F.—a comparatively low temperature for efficient disinfection. Legge concludes that, to secure certain destruction of all anthrax spores in horsehair, absolute reliance cannot be placed on either steam disinfection (within the limits in which it can be applied) or simple boiling.

In the Home Office Regulations in respect of processes involving the use of horsehair from China, Siberia, or Russia, no manipulation except opening or sorting is allowed until the hair has undergone disinfection. Disinfection may be accomplished either by exposure to a temperature of not less than 212° F. for at least half an

hour, or by exposure to a disinfectant which has been certified as effective for the destruction of anthrax spores, and approved by the Secretary of State. Eurich, the bacteriologist to the Anthrax Investigation Board, said (1909) that no trustworthy method of sterilising the wool before washing had been found. In 1913, Eurich and Willey conducted experiments in regard to the use of steam as a disinfectant, as a result of which their Board agree that disinfection by steam cannot be applied to ordinary wool or hair except under conditions that would stop any trade in the sorts so treated. The method can, however, be applied to blood-stained material that has been sorted out or otherwise separated from the bulk, so that blood-stained material need no longer be regarded as absolute loss, but as a waste product.

A process of destroying carcasses involves solution of the entire animal in sulphuric acid. The Anthrax Order of the Board of Agriculture (1911) provides for prosecution for causing an effusion of blood, and no one may burn, bury, or otherwise dispose of the carcass without the sanction of the local authority.

The Tetanus Bacillus.

Morphology.—*B. tetani* is a straight, slender, rod 4 μ long, with rounded ends. It is slightly motile, possessing many peritrichic flagella. Especially in old cultures, long filaments are formed which show no spores. Spherical spores are frequent, which, being located at one end, and being thicker than the bacillus, gives the 'drumstick appearance.' The bacillus is Gram-positive.

Culture.—The tetanus bacillus is a 'strict' anaërobe, although Jordan states that a degree of tolerance to oxygen can be established, and that it will grow in a mixed culture when air is admitted. In glucose-gelatin or glucose-agar stab culture, a feathery radiated growth is formed with slight gas-formation. Gelatin is liquefied. All cultures possess a characteristic smell, suggestive of an ill-kept stable. The organism grows well on blood-serum without liquefaction.

Theobald Smith has shown that the bacillus will grow in ordinary broth in a fermentation tube if a piece of sterile tissue, such as a piece of liver, kidney, or spleen of guinea-pig or rabbit, be inserted where open bulb and

closed arm meet. No precaution for exclusion of oxygen is necessary, and spores are said to develop in a day or little longer. Embleton says *B. tetani* will grow in broth along with *Staphylococcus aureus* and need no precaution to exclude oxygen.

Tetanus spores, especially if in a dry condition, keep their virulence for an indefinite time. Much uncertainty exists as to the temperature necessary to kill them. It seems probable that sometimes they withstand boiling for five minutes and require heating in an autoclave for their certain destruction. Embleton says they can stand almost a dull red heat. The resistance of the spores to disinfectants is also high. They survive 5 per cent. phenol solution for fifteen hours.

Channels of Infection and Pathogenesis.—The tetanus bacillus and its spores are found very frequently (90 per cent. of samples) in cow and horse dung and less rarely in human fæces (5 per cent. of samples, Andrewes). It is constantly present in manured land. Unless some degree of penetration into the tissues is effected, the disease does not develop. If only a scratch, abrasion, or superficial cut be suffered, infected material obtaining access thereto seems never to become pathogenic. If manured soil, however, be injected into a laboratory animal, or a stableman or gardener gets wounded with a rusty nail or broken glass, the disease may be produced. The disease is more prevalent in some districts than in others, and by following the statistics of French veterinary surgeons, Bazy (*Medical Press*, 1915, p. 315) has been able to mark out certain 'accursed fields' for tetanus just as can be done for anthrax. The disease is prevalent among horses and men on battlefields. The incidence varies with the state of the ground fought over. In the present war the disease is non-existent among troops engaged on uncultivated areas round the Dardanelles just the same as there has been no tetanus in naval cases of wounds. On the highly cultivated grounds of Flanders and France, especially in the Aisne region, cases were not infrequent. Bazy's statistics bear on 10,396 wounded; of this number 129 developed tetanus, of which 90 proved fatal.

Umbilical tetanus is common among newborn infants in some countries. Puerperal tetanus and tetanus supervening after operations are now rare. Impure vaccine

lymph and Fuller's earth have occasioned the disease. The spores can on occasion live through the operations involved in the manufacture of gelatin, and injection of contaminated gelatin has produced tetanus.

The organism is pathogenic to man, the horse, guinea-pig, mice, and rabbit; birds are but slightly susceptible. The bacilli remain in the locality of the site of inoculation and in the nearest lymph glands, symptoms being caused by absorption of the toxins. In the development of the disease, damage to local tissues and prevention of phagocytosis are the most important factors, the latter being allowed by the extraneous organisms which gain access with the tetanus bacilli. The spore, if freed from toxin by simple washing, is readily disposed of by phagocytosis.

Usually fifteen days elapse between infection and the appearance of symptoms, but they may occur in man in two days, or not till after twenty-seven days.

It is rarely that drumstick spores can be found in material from a wound, and should any be found, the existence of other Gram-positive bacilli growing terminal spores must not be overlooked.

Toxins.—Grown anaërobically in glucose broth, the tetanus bacillus produces a powerful extracellular toxin, of the constitution of which little is known. There is a substance producing the characteristic spasm (tetanospasmin), a hæmolysin (tetanolysin), and some albumoses to which Sidney Martin ascribes the fever of tetanus. The toxin is readily destroyed by heat and light, and diminishes in toxicity with keeping. Tetanus toxin is rapidly fixed by the tissues of the central nervous system, and travels from the wound by way of the nerve trunks.

Antitetanic Serum.—In Roux and Vaillard's process, virulent tetanus bacilli are cultivated in broth in an atmosphere of hydrogen. After about fourteen days' growth, the culture is filtered through a porcelain filter to free it from bacilli. Injections are then made into horses with this toxin daily, subcutaneously or intravenously, starting with 1 c.c. of iodised toxin, gradually increasing the dose until the pure toxin can be injected without danger, the treatment lasting about three months. The serum is standardised. The results attending the use of this serum have been less satisfactory than those obtained with antidiphtheria serum, as the disease is only

recognised when considerable absorption of toxin has occurred. Its use as a prophylactic is undoubted. Even in those cases where the disease is firmly established the serum is often still of service. In field ambulances, etc., where surgeons systematically give prophylactic injections, Bazy says the mortality is only one-third of that where it is only given to suspicious cases.

The Bacillus of Malignant Œdema.

Morphology.—*B. œdematis maligni* is a motile rod, about 4 μ long and 0·1 μ broad, with several flagella. It has a tendency to grow in long filaments. It forms spores in a more or less central position, but not when in filamentous form. It stains readily with the ordinary dyes, but is Gram-negative.

Culture.—Distinction from the anthrax bacillus is also seen on cultivation. It is a strict anaërobe, and grows at room temperature. Development is accelerated by the presence of 2 per cent. of glucose. Gelatin is liquefied, and a foul-smelling gas is produced in both gelatin and agar stab cultures. Blood-serum is liquefied, but there is no visible growth on potato.

Distribution and Pathogenesis.—Malignant œdema has followed subcutaneous injection of musk, and the organism has been found in musk-sacs. It may follow castration in the horse or parturition in cattle. The organism occurs in the soil, dust, and in the human intestine.

The bacillus may be the exciting cause of gangrene after injuries, particularly when the parts are crushed or lacerated and soiled with earth, and is pathogenic to man, horses, pigs, sheep, guinea-pigs, rabbits, rats, mice, and some birds. The readiest plan for isolation is to inoculate subcutaneously a guinea-pig with garden earth. On death, which may occur in twenty-four to forty-eight hours, the bacillus is found in the œdematous fluid, but not, like anthrax, in the blood, except later, when it has multiplied after death, when it may form filaments 15 to 40 μ in length.

The gas manifested in the frothy exudation when an animal is inoculated with garden earth is absent, or nearly so, when the inoculation is made from a pure culture, and is therefore probably due to other organisms.

Bacillus (Clostridium) Chauvœi.

Black-leg, quarter-evil, or symptomatic anthrax is unknown in man, but in sheep and oxen a fatal termination is to be apprehended one or two days after infection. Localised swellings appear on the neck, shoulders, or thighs, and the affected muscles become discoloured. Puncture of infected muscle produces a frothy, sanguineous fluid, which contains the organism. The organism is a rod about 4 μ long, and is actively motile. The large spores give it club- and spindle-shaped appearances. It is a strict anaërobe, and is usually Gram-negative. It grows in agar and gelatin cultures with the production of a foul gas. Gelatin is slowly liquefied. Kitt says the odour is due, not to *B. Chauvœi*, but to contamination with 'cadaver bacilli.' By drying an affected muscle at 35° C. and then heating to 85° or 90° C., attenuation in virulence is effected, and the material is injected for the production of immunity.

Red Braxy in sheep is associated with an organism similar to *B. Chauvœi*, which, however, differs in its action on other animals. The bacilli are very numerous in the intestine and in the reddish-brown blood-stained mucus that collects in the last and true digestive stomach. After death, decomposition is very rapid, and the crimson colour of the fourth stomach is sometimes mistaken for a symptom of irritant poisoning. Hamilton found that during July and August the serum of healthy sheep did not allow multiplication of braxy bacilli. Very satisfactory immunity is obtained by dosing lambs by the mouth when their blood is most refractory to the organism.

Bacillus Botulinus.

Morphology.—A large bacillus (4 μ to 10 μ long), with rounded ends, and a tendency to form short chains. It is an obligatory anaërobe, exhibits a slight motility, and forms spores. The spores are often destroyed by a temperature of 80° C. The position of the spores is generally terminal, and the flagella number from four to eight. The organism is Gram-positive.

Cultural Characters.—Cultures must be grown in the dark. Milk is not curdled; glucose is fermented with production of acid and gas; saccharose and lactose are

not fermented. While Stockman mentions that cultures of this organism have no putrefactive odour, other observers describe a rancid odour due to butyric acid. A stab culture in glucose gelatin grows white along the stab with lateral offshoots, liquefaction and disruption of the medium with gas. On glucose-gelatin plates the young colonies are translucent, of a yellowish-brown colour, and surrounded by a liquefied zone. Growth is abundant at ordinary temperatures, but scanty at 37° C., at which temperature involution forms of long twisted filaments develop. Sporulation does not occur at blood-heat.

Pathogenesis.—The organism has been found in liver and blood sausages, pickled and smoked meat, tinned meat, preserved foods, and ham that have caused 'botulism' or 'sausage poisoning.' Where symptoms show immediately after ingestion of the infected food they are those of gastro-intestinal disorder. It is not till after a period of incubation of twelve or more hours that the prominent symptoms described by van Ermengem show, these being disordered secretion in the nose, dryness of mouth and throat, dilated pupil, ptosis, paralysis of accommodation, double vision, dysphagia, aphonia, and retention of the urine. Fever is absent, and no impairment of intellect occurs. While marked constipation has been recorded, there may be slight and transitory vomiting and diarrhœa. Death, from bulbar-paralysis, follows in about a quarter of the cases.

Except for an instance where a bacillus indistinguishable from *B. botulinus* was isolated by Kempner from the intestine of a pig, there is no information as to the origin of the organism. From the scanty growth obtained at blood-heat it is fairly certain that little or no multiplication of the bacilli takes place in the living animal, so that a sufferer is not a source of danger to his associates, and it is pretty evident that infection of food must have occurred after death of the animal, the infection coming from sources outside the meat. Savage in reviewing the reported cases of botulism found that in none of the outbreaks had the foods incriminated been eaten in the fresh state, all had been stored. There is often no external sign of putrefaction in the infected meat, but it may have a rancid odour.

An extracellular toxin of extremely high potency is

formed by this organism, and the intoxication is due to its formation in meat before ingestion. The toxin is destroyed by exposure to 80° C. for half an hour, and food causing the outbreaks has been either raw or imperfectly cooked. As growth of the bacillus is checked by a 6 per cent. solution of salt, van Ermengem has recommended that in salting a brine containing at least 15 per cent. of sodium chloride should be used.

The disease is now rare on the Continent, and Savage was unable to trace the occurrence of a single outbreak in this country.

An antitoxin prepared by Kempner is said to be of value even some hours after ingestion of infected food.

Bacillus Welchii.

An organism commonly found in emphysematous gangrene was discovered by Welch and Nuttall (1892), and named *B. aerogenes capsulatus*. Fränkel (1893) independently described *B. phlegmones emphysematosæ*, which is identical with Welch and Nuttall's organism. Klein attributed an outbreak of diarrhœa in St. Bartholomew's Hospital (1895) to an organism (*B. enteritidis sporogenes*) found by him in the dejecta of patients. Oftentimes, regardless of all the attributes of Klein's bacillus, this name is commonly applied to a normal inhabitant of the digestive tract which is described here as *B. Welchii*. Other observers have independently discovered organisms either identical or very similar. Chester has renamed this organism, or class of organisms, *B. Welchii*.

Morphology.—*B. Welchii* is a plump, long bacillus (3 μ to 6 μ long), with almost square ends, occurring singly, in chains, and in clumps. The length varies greatly on culture, when different media are used. It frequently has a capsule, and forms oval spores, generally situated near one end, but only in serum cultures. It is strictly anaërobic, is Gram-positive and non-motile. *B. enteritidis sporogenes* is described by Klein as multi-flagellate and motile, and as sporing in gelatin.

Cultural Characters.—Gelatin is slowly liquefied. Gas (two-thirds or three-quarters of which is hydrogen) is produced in dextrose, lactose, and saccharose media. Since its growth is not suppressed by bile-salt it gives a positive reaction in MacConkey's bile-salt media like the

colon bacillus does. A characteristic fermentation is produced in milk in forty-eight hours: a firm, white, honeycombed curd and a clear, watery whey are produced with abundant gas-formation. The culture is strongly acid, and has a faint, sour smell, like that of butyric acid. Most varieties liberate hæmoglobin when grown in broth containing blood (Jordan). When grown anaërobically in undiluted human blood serum with staphylococcus, very copious sporing occurs in two days (Douglas). Fleming recommends neutral-red egg medium for surface growths: in twenty-four hours bright pink spots, 2 millimetres in size, appear, having slightly raised margins and characteristic prominences in the centres.

Occurrence.—*B. Welchii* is found in excrementitious matter and road dust. Its presence in water, milk, shellfish, etc., is to be regarded as an indication of pollution, not necessarily recent, with fæcal matter.

Pathogenesis.—Subcutaneous inoculation of guinea-pigs with recent milk cultures produces in some cases intense spreading hæmorrhagic œdema and necrosis, and death in twenty-four to forty-eight hours. In other cases it is not pathogenic.

Rabbits are almost immune, but if one be inoculated intravenously, killed, and the carcass incubated at 37° C. for twenty-four hours, characteristic production of gas follows, especially in the liver (Welch-Nuttall test). *B. Chauvœi* produces a similar result, but forms spores, while (according to Hewlett) *B. Welchii* does not under such conditions.

While the *B. Welchii* is either absent or rare in the fæces of the young, Kendall has found it in the stools of children with summer diarrhœa. Hewlett thinks it is probably capable of producing necrotic changes in the intestinal mucous membrane, and, because of its abundance in the intestine in some cases of primary anæmia, suggests it may have some relation to the condition. It occurs in various pathological conditions, in septicæmic and pyæmic infections of the gastro-intestinal and genito-urinary tracts, and is often responsible for the gas seen in 'foamy organs' at autopsies.

In cases of gaseous gangrene occurring among our troops in France *B. aerogenes capsulatus* (*i.e.*, *B. Welchii* as originally described by Welch and Nuttall) is dominant in every case. Quinine hydrochloride is found to reduce

the mortality considerably in experimental animals (Kenneth Taylor, *Lancet*, September 4, 1915). The same organism has been found in the expectorated matter from cases of pulmonary gangrene supervening after inhalation of the irritant gases employed by the Germans (De la Riviere and Leclercq, *Medical Press*, October 20, 1915).

Detection in Water.—*B. Welchii* is regarded as of less importance as an index of pollution in water than formerly. Large quantities of water must be examined, and it was previously the custom to pass half a litre through a Pasteur-Chamberland candle, suspend the deposit in 5 c.c. of sterile water, and inoculate three milk tubes with 3 c.c., 1 c.c., and 1 c.c. respectively of the concentrated water. The milk tubes were then treated as described below. Hewlett's method of conducting the test, though cumbersome, is more satisfactory. Ten large boiling tubes, each containing 50 c.c. of sterile milk, are inoculated each with 50 c.c. of the water, melted vaseline is poured on the surface of the mixture to exclude air, and each tube is covered with a double layer of sterile filter-paper, kept in position by a rubber band. The tubes are heated in a bath of water to 80° C. for twenty minutes, and incubated at 37° C. for two days. When smaller amounts, such as 10 c.c. of liquid, are to be examined, an exposure to 80° C. of ten minutes is sufficient. The gas produced in the fermentation (*vide supra*) collects as a bubble under the vaseline plug. The *Clostridium butyricum* (which is principally responsible for the butyric fermentation of milk, and which, when present, hastens the production of tyrotoxicon therein) gives a similar reaction in milk, but is not pathogenic for guinea-pigs. The only way to distinguish *B. Welchii* from *Clostridium butyricum*, which is regarded by some as a non-pathogenic form of the former, is to inject 2 c.c. of the whey subcutaneously into a guinea-pig of about 200 grammes weight, when *B. Welchii* will kill the animal in forty-eight hours.

To isolate *B. Welchii* from pus, Alexander Fleming (*Lancet*, August 21, 1915) recommends the following procedure: A tube of sterile milk is boiled and immediately, while yet at a temperature close to 100° C., is planted with some of the pus. The scum of cream suffices to exclude air. If other spores get in, anaërobic plate cultures on glucose agar are made.

CHAPTER VII

THE COLON-TYPHOID GROUP

This group of bacilli is classed by Löffler as one family, the *Typhaceæ*. The characters of individual members are not always clearly defined. As a general rule the organism is short, plump, with rounded ends, often with a tendency to develop long forms. No spores are formed and the organisms are Gram-negative. None liquefies gelatin except *B. cloacæ* and a few coliform organisms described by MacConkey (*B. levans, B. oxytocus perniciosus*, and his No. 73). On a gelatin plate, they form thin, irregular, notched colonies. All are aërobes and facultative anaërobes, none are chromogenic, and all grow well on ordinary media.

The Colon Bacillus.

Bacillus coli (*B. coli communis*) inhabits the intestinal tracts of man, the lower animals, birds, and, less frequently, fish. The dispersion of the dejecta causes this organism to be widely distributed. While a very large proportion of the organisms when isolated are found to conform to the salient characteristics of the recognised type, 'atypical' organisms are met with which, in one or more respects, differ from the true type. Such are described as 'coliform.'

Morphology.—The colon bacillus is a short rod, 2 μ to 4 μ long, with rounded ends, but may form much longer rods, or be so short as to be oval in shape. It possesses three or four flagella on an average, and although generally feebly motile, non-motile forms are not uncommon. It forms no spores, and is not stained by Gram's method.

B. coli is slightly more resistant to heat, to disinfectants and other destructive agents than is *B. typhosus*. While *B. coli* does not usually survive an exposure to 60° C. for ten minutes, one variety, *B. Grünthal*, produced a toxin that withstood ten minutes at 80° C. According to Ayers and Johnson 54·6 per cent. of cultures survived thirty minutes at 60° C., and 6·9 per cent. survived 62·8° C. for thirty minutes.

Cultural Characters.—On gelatin plates colonies appear in one or two days, the growth being thin and filmy, irregular in shape, translucent at the margins, and moist in appearance. In gelatin stab or shake cultures bubbles of gas are produced. The gelatin is not liquefied.

On serum and on agar greyish-white, moist, shining growths form. On potato, if acid, a yellowish growth is obtained, but the growth may be colourless if the potato is not fresh. Milk is rendered acid, and curdled in from one to three days at 37° C. without subsequent digestion of the casein and without subsequent production of alkalinity. When grown in peptone-water containing one of the following substances—glucose, lævulose, maltose, galactose, arabinose, raffinose (?), lactose, mannite, sorbite, dulcite or dextrin (cane-sugar, sometimes)—acid and gas are produced. The gas production varies in amount. From the fact that an organism may lose its power of forming gas from sugars while retaining its power of forming gas from alcohols, Penfold concludes that two enzymes are concerned (see also p. 28). The gas produced from dextrose consists of hydrogen 2 volumes and carbon dioxide 1 volume. Present American opinion places no reliance on this formula. *B. coli* produces indole in broth or peptone-water cultures, generally within two days, but sometimes a week is necessary before it can be demonstrated. It reduces nitrates to nitrites. In neutral-red lactose media the acid formed produces a rose-red colour. On solid media no further change of colour takes place, but in neutral-red lactose broth, the dye is reduced with production of a greenish-yellow fluorescence.

In the Voges-Proskauer reaction the organism to be tested is grown for three days in 2 per cent. glucose broth. Two or 3 c.c. of strong caustic potash are added, and a pink eosin-like colour developing on exposure to air for twenty-four hours constitutes a positive reaction. The colon bacillus does not give this reaction. Rivas devised a quick method of performing this test: 1-4 c.c. of a forty-eight-hour culture is boiled with 5 c.c. of 10 per cent. sodium hydrate solution, the reaction being hastened by blowing air through the liquid or by shaking.

By means of fermentation and other reactions over a hundred types of colon bacilli can be distinguished. It

becomes, therefore, necessary to enunciate definitely those attributes which, if possessed by an organism, will entitle it to be regarded as a colon bacillus. Of course when a pathogenic organism of this class is isolated from a patient, the name it is called by in the present state of knowledge matters little so long as it prove *corpus delicti*. But when estimating the organism to ascertain existence of fæcal contamination a reasonable degree of definition is necessary. Different bacteriologists select different characters to which they require an organism to conform before identifying it as typical *B. coli*. Houston described an organism as 'flaginac' if it produced a fluorescent greenish yellow in neutral red glucose peptone-water (*fl*), acid and gas in lactose (*ag*), indole in peptone-water (*in*), and acid and curd in milk (*ac*). MacConkey suggests the omission of tests for the growth on gelatin, action on milk, glucose, and neutral red, and the indole test. In the place of them he recommends the action of the organism on (1) dulcite, (2) adonite, (3) inulin, and (4) the Voges and Proskauer reaction.

A negative indole reaction becomes more significant when the para-dimethyl-amido-benzaldehyde test has been used instead of nitrite and acid.

It is possible, but by no means certain, that certain varieties of the colon bacillus are of greater significance as indicators of excretal contamination than others. The presence or absence of power to ferment saccharose is particularly thought worthy of emphasis in this connection, and Houston denominates those fermenting saccharose, producing acid and gas in lactose peptone water, and giving an indole reaction as 'sagin' organisms. Those having no action on saccharose, but giving the other two tests, are similarly christened 'agin' bacilli. The bacillus producing acid and gas from cane-sugar (saccharose) has also been called *B. coli communior* by Durham, and this variety is supposed to be of more recent intestinal ancestry than another not fermenting saccharose.

The American Committee mention the following attributes as defining the colon group: 'Fermentation of dextrose and lactose with gas production, short bacillus with rounded ends, non-spore-forming, facultative anaërobe, gives positive test with esculin, grows at 20° on gelatin and at 37° on agar, non-liquefying in fourteen days

on gelatin. Gram-staining negative.' Prescott and Winslow doubt there to be 'sufficient evidence to warrant making the esculin reaction a general criterion of the colon group,' and they desire to reduce the test to positive reactions in a lactose fermentation medium, growth on an aërobic agar streak, and microscopic examination.

Clemesha and Houston have independently shown that with fresh fæcal pollution in water most of the organisms fermenting dextrose ferment lactose as well, while in stored or filtered water dextrose-fermenting lactose-negative forms relatively increase.

To obtain presumptive evidence of the presence of the colon bacillus a medium containing a suitable carbohydrate and some substance to inhibit the growth of other organisms is used, such as MacConkey's litmus lactose bile-salt peptone-water, which is fermented with the production of acid and gas. This medium is used for further work (see p. 230).

If the organism be present in large numbers and vigorous condition, acid and gas production will be evident in less than twenty-four hours, but attenuated organisms may require three days' incubation at blood-heat or at a slightly higher temperature. Bile-salt has the disadvantage of suppressing some of the weak coliform organisms. In examinations for fæcal contamination this is not a matter for worry, as feeble vigour *cæteris paribus* denotes remoter fæcal ancestry, and the attribution of slighter significance to their presence and numbers. (It may be mentioned here that the best 'pick-me-up' media for rejuvenating weak bacteria are liver broth, and gelatin at blood-heat.)

Pathogenesis.—While in the intestine, the colon bacillus is supposed by some to suppress the growth of less desirable organisms, and thus to protect the body. This surmise is based on the diminution in number or absence of the colon bacillus from the fæces in some bacterial diseases affecting the intestines. Although chiefly of importance as an 'indicator' of pollution with excrement, the colon bacillus is also capable of causing disease. The organism is pyogenic, is commonly the cause of cystitis, and may affect the gall-bladder and bile ducts and other organs. Sir Almroth Wright suggests it may sometimes be responsible for mucous colitis. Arnold Lawson

describes cases of metastatic ocular inflammation as associated with *B. coli* toxæmia. It is generally the causal agent of calf diarrhœa (Bibby's 'Bovine Tuberculosis,' p. 434). It is possible that *B. coli* may be responsible for some outbreaks of bacterial food poisoning. Gordon R. Ward thinks a variant of *B. coli* produces pernicious anæmia.

Bacilli coli as pathogenic organisms are supposed not to be identical with, but relations of the original *B. coli;* to use Metchnikoff's phrase, they are 'wild races.' Some bacteriologists drop the term *B. coli* when speaking of an infective organism of the species and speak of it as a coliform organism.

Vaccines.—Antisera have not proved of value. Cystitis and other affections of a chronic nature are successfully treated with autogenous vaccines. Greater improvement takes place when the vaccine is freshly prepared every month.

By cultural characters alone, over a hundred types of *B. coli* have been described. As organisms culturally identical give widely different agglutination reactions it is quite evident that more types still undescribed exist. Even allowing for many types that are incapable of producing disease there must remain an enormous number of potentially pathogenic colon bacilli. In coli infections it remains largely a matter of chance whether a stock polyvalent vaccine will be antagonistic to an individual pathogenic type.

Coli-like Organisms.

In addition to the atypical *B. coli* mentioned, other organisms have been regarded as variants of the colon bacillus. Some of these are mentioned among the capsulated bacilli (*vide infra*).

B. neapolitanus, isolated by Emmerich from the dejecta of cases of cholera, ferments saccharose and dulcite, and produces indole, but is non-motile.

B. cloacæ, a motile organism, liquefies gelatin quickly or slowly, ferments saccharose but not dulcitol, and gives the Voges-Proskauer reaction. It occurs in sewage, graveyards, and slaughter-house drains.

B. bifidus and *B. acidophilus* are coli-like, but Gram-positive organisms, which occur in the large intestine of

human infants. They are described with *B. bulgaricus*, with which Rodella thinks them identical (see p. 189).

B. coli anaerogenes, an organism of the coli-type, but producing no gas in the fermentation reactions. Is distinguished from *B. typhosus* by its production of acid in lactose media and by agglutination reactions.

B. acidi lactici (pp. 188 and 108) is occasionally pathogenic.

The Capsulated Bacilli.

B. pneumoniæ, or Friedländer's pneumo-bacillus (see p. 143).

B. lactis aerogenes, found in the bowels of nurslings and sometimes in souring milk, is non-motile, does not ferment dulcitol, and gives the Voges-Proskauer reaction. In gelatin a projecting 'nail-head' growth is formed, and milk is usually curdled more rapidly than with *B. coli*, with the formation of capsules. It is sometimes pathogenic.

A short capsulated bacillus has been described in rhinoscleroma, which is Gram-negative, does not liquefy gelatin, and does not curdle milk.

Several organisms have been described in ozæna (fœtid atrophic rhinitis), one of which — Abel's *B. ozænæ*—is capsulated, and causes the atrophy of the mucous membrane.

Bacillus Enteritidis (Gärtner).

Morphology.—An actively motile bacillus, with shape and size similar to those of the typhoid bacillus, carries several flagella, forms no spores, and is Gram-negative.

Cultural Characters.—Ferments glucose, lævulose, maltose, galactose, arabinose, raffinose, mannite, sorbite, dulcite and dextrin, with production of acid and gas. Has no action on saccharose, salicin (as a rule), nor glycerin (as a rule). It is generally regarded as without action on lactose, but some strains attack it. Neutral red is reduced with production of yellowish fluorescence. Milk is not curdled. In litmus milk in the first day a slight acidity develops which is replaced by a permanent alkalinity. The bacillus gives very little or no indole, and does not give the Voges-Proskauer reaction. Identification as the cause of disease may be shown as in the

case of the paratyphoid bacilli by a satisfactory agglutination with the patient's serum.

Pathogenesis.—Gärtner's bacillus is the most frequent cause of epidemics of meat poisoning. It has been discovered in meat from pigs, cattle, horses, and fish. In the only case of infection in mutton, the meat was supposed to have been infected from contaminated ox tongue (the organism found being *B. suipestifer*). In many cases meat has been derived from animals that had enteritis, in other cases all the evidence points to infection of the meat *post mortem*. It is known that a piece of infected meat will infect a sound piece if in juxtaposition.

It has been suggested that Gärtner bacilli are more or less natural inhabitants of the animal intestine. Savage has shown that, although prepared meat foods are very often subject to extensive contamination of excretal origin, Gärtner bacilli are not often to be found. He is therefore of opinion that the Gärtner Group organisms (or other special bacilli) are ' derived from animals which are either at the time suffering from disease due to Gärtner Group bacilli, or acting as carriers of these bacilli.'

Infected meat has usually been normal in appearance, taste, and smell, but salty or other peculiar flavours have been sometimes noticed, and turbidity of jelly was mentioned in one case.

Gärtner's bacillus is easily killed by heat, not surviving thirty minutes at 60° C., but it produces a heat-resistant toxin that, as it is not destroyed in thirty minutes at 100° C., would survive most cooking processes.

Symptoms are due to action of the toxins, their onset is generally sudden and may include vomiting, diarrhœa, pains in abdomen and head (sometimes in back and limbs as well), prostration, collapse, cold sweats, rigors, cramps, rashes, furred tongue, offensive breath and moderate pyrexia.

If the affected food contain toxins preformed in the food, onset of symptoms may take place almost immediately after ingestion. In most cases, however, the food contains toxins and bacteria, and symptoms may commence any time within thirty-six hours. Bainbridge suggests the rat's intestine to be the true home of Gärtner's bacillus (see also p. 97).

Organisms Resembling Gärtner's Bacillus.

Para-Gärtner Bacilli (or pseudo-Gärtner bacilli).—Savage has described a number of organisms in the healthy human and animal fæces, that can only be distinguished from true Gärtner bacilli by extended schemes of fermentation reactions and by their failure to agglutinate with sera from animals immunised to Gärtner bacilli.

Bacillus Paratyphosus β.—The paratyphoid bacillus β is similar to *B. enteritidis* (Gärtner) in cultural reactions, and, like the latter, produces a toxin that withstands 100° C. for thirty minutes. While generally producing symptoms like those of a mild typhoid infection, it has been held responsible for some meat-poisoning outbreaks. Savage, while not disputing the possibility of this organism causing food-poisoning outbreaks, showed that where *B. paratyphosus* β has caused acute gastro-enteritis simulating a food-poisoning outbreak, the cases were connected, not by a common food supply, but by contact with a source of infection probably human. In the Cholet outbreak, Chantemesse found the bacilli in the confectioner's cream supplied for the wedding breakfast, and ascertained that the confectioner was a paratyphoid carrier.

Bacillus Paratyphosus α.—Like the β bacillus, this organism produces symptoms resembling mild typhoid, but it is much less frequent than the β bacillus.

In their reactions the paratyphoid bacillus *a* stands nearer the typhoid bacillus than does the paratyphoid bacillus β. Group *a* produces less gas in glucose media than group β. Milk remains permanently acid with group *a*; with group β it becomes alkaline after an initial acidity. Though group *a* changes neutral-red to yellow, the red colour returns after three weeks or so, while with group β the yellow colour is permanent.

In paratyphoid fever, whether caused by *a* or β bacilli, the organism may be found in the blood and fæces. The mortality is low (less than 3 per cent.), and in the autopsy the characteristic typhoid ulcerations of the Peyer's patches are absent. Milk and meat are supposed to have conveyed the infection, and the organisms have been isolated from water.

In identification of the disease and of any suspicious organisms isolated, agglutination tests are important. Paratyphoid serum either fails to clump *B. typhosus* or only does so in low dilution, but agglutinates the appropriate paratyphoid bacillus in dilutions of over 1 in 100. Examination of the fæces is performed as for typhoid.

At the time of writing paratyphoid appears to be more frequent in the services than typhoid, often occurring as a chronic dysenteric condition. The diseases are frequently as severe as typhoid. Anti-enteric vaccine is frequently a mixed preparation of typhoid, paratyphoid *a* and paratyphoid *β* bacilli. This gives protection against any or all of the three diseases. A triple infection with the three diseases has been described (Castellani).

Bacillus Psittacosis.—Imported parrots are liable to an infectious enteritis with septicæmia (psittacosis) which is communicable to man, producing a fatal bronchopneumonia. The psittacosis bacillus belongs to the Gärtner group, and is partly clumped by typhoid serum, some bacilli between the clumps retaining motility.

Bacillus Suipestifer (B. Suicholeræ).—This organism was formerly regarded as the cause of swine fever. The disease is now known to be due to an ultra-microscopic organism, but *B. suipestifer* always seems to invade the tissues of animals with swine fever (hog cholera), and is credited with pathogenic properties for that animal. Its presence in meat has caused food poisoning. *B. suicholeræ* is supposed to be identical with Sanarelli's *B. icteroides* and with *B. Aertryck*. While German authorities consider *B. suipestifer* to be identical with *B. paratyphosus β*, Englishmen maintain them to be distinct organisms.

Rat and Mouse Viruses.—The Danysz bacillus, cultures of which ('Danysz virus') are sold for the extermination of rats, belongs to this group, as do the *B. typhi murium*, used as a mouse virus, and organisms found in other makes.

Bainbridge showed that the viruses owe their potency to bacilli indistinguishable from *B. Aertryck* and *B. enteritidis*. Their destructive power on rats was inconstant, the death rate varying from 20 to 50 per cent. Some of the rats recover and become immune, while others do not even suffer at all. The use of rat viruses appears to be sometimes attended with dangerous results.

They are one and all certified to be harmless to human beings, and in some cases this is based on actual experiment. As Bainbridge says, the entire innocence of the bacterial viruses for man is a statement which needs justifying.

Collingridge attributed an outbreak of illness lasting forty-eight hours—and characterised by headache, giddiness, and cramp—to a virus used for killing mice in a London business establishment. Nine girls at an Irish convent school died, and McWeeney attributed the outbreak to *B. enteritidis* in beef, which, he said, was possibly infected by sick mice in the larder.

Savage and Read found true (Gärtner) *B. enteritidis* in the spleen and liver of apparently healthy rats and regarded its presence as the result of an old infection from which the rats had recovered. Savage thinks that there is a strong probability that rats surviving an epizootic of this nature will act as carrier cases for some time.

Morgan's No. 1 Bacillus.—While this, or a very similar organism, is to be found in the normal stools of children occasionally, it is much more frequent in the motions of summer diarrhœa of children, and is accepted as being frequently the cause of this condition. It produces acid and a little gas in dextrose, but does not ferment lactose, saccharose, nor mannite. It does not liquefy gelatin nor give the Voges-Proskauer reaction, but it produces indole and slowly turns milk alkaline with no primary acidity. It has been found on flies in houses with cases of summer diarrhœa.

Diseases of Calves.—Gärtner group bacilli are responsible for or present in some cases of septicæmia, dysentery, pneumonia, and other septic diseases of calves. Jensen's paracolon bacillus of calf dysentery belongs to the group.

The Typhoid Bacillus.

Morphology.—*B. typhosus* (the Eberth-Gaffky bacillus) is 2 μ to 4 μ long by 0·5 μ thick, with rounded ends, but shorter, longer, and, occasionally, filamentous forms are seen in cultures. No spores are formed, but granules and vacuoles may be seen. Involution forms 10 μ to 30 μ long are obtained on repeated subculture, and are somewhat characteristic. The organism is aërobic and

facultatively anaërobic and Gram-negative. It is not killed by drying; its thermal death-point is 55° C. Diffused daylight prevents its development, and direct sunlight is fatal in five hours.

The typhoid bacillus usually carries eight to twelve flagella arranged round its sides and end. It is actively motile, and in hanging-drop preparations some of the organisms are seen to progress at a surprisingly rapid rate, while others rotate rapidly. While growth is best at blood-heat, the bacillus develops well at room temperature, though less quickly than does the colon bacillus.

Cultural Characters.—On agar the typhoid bacillus produces a thick, greyish, creamy layer. On gelatin it gives a white, thin growth, usually almost confined to the needle track, and without liquefaction. In a gelatin shake culture the growth forms a diffuse haziness, without any gas-bubbles. The surface colonies in gelatin plates are small (1 millimetre), semi-transparent, bluish-white in colour, with an irregular outline; the deep colonies are roundish points with sharp margins, finely granular, and yellowish-brown in colour. In broth a general turbidity is produced, with some deposit, but no pellicle. Milk is rendered slightly and permanently acid, but there is no curdling. Occasionally alkalinity may succeed acidity if culture in milk be prolonged. On slightly acid potato a thin, moist, greyish, almost invisible layer is formed. Glucose and mannite are fermented with the production of acid only, no gas, but lactose and saccharose are unattacked. It forms no indole, does not give the Voges-Proskauer reaction, and neutral red is unaltered.

Strains of typhoid bacilli showing slight divergence in some cultural character from typical attributes are met with. We found a carrier strain to slowly ferment lactose with production of acid (in three days) (see also p. 28).

Strains grown on lactose, dulcite, or arabinose acquire the power of fermenting the respective carbohydrate or alcohol to which they have been 'educated,' but many educated strains quickly revert to the non-fermenting typical organism.

Channels of Infection—*Water.*—Although water contaminated with sewage may be drunk with impunity, should the dejecta of a case of typhoid contribute to the pollution, an epidemic of typhoid may be expected. In

many cases infection has been traced to water which chemical analysis showed to be of considerable organic purity. Even bacteriological examination in which colon bacilli and streptococci have been estimated will not necessarily reveal danger from typhoid. Where a previously pure water is contaminated with urine from a typhoid carrier, typhoid bacilli will be present without colon bacilli and streptococci. Therefore, while as a rule the 'bacterial indicators of pollution' (normal intestinal inhabitants) are to be found in a typhoid-bearing water, it does not follow that because they are absent, typhoid bacilli are also absent.

Bacteria are not evenly distributed through the bulk in unfiltered water, many being aggregated in masses, so that where typhoid infection is present some of the bacilli will be discrete, but very many will clump together. Consumers of this water only imbibing separated bacilli are not likely, or are less likely, to acquire the disease. But the consumer who receives a clump of typhoid bacilli is the less likely to resist infection (spotted distribution). Storage and filtration, Houston believes, remove all likely danger from aggregated microbes, and tend to render separated microbes fairly equally distributed throughout a water supply.

There is no ground for the suggestion that *B. coli* may become transformed into the typhoid bacillus. The persistence of the typhoid bacillus in water is largely governed by the chemical constitution and bacteriological characters of the medium (see also p. 225).

Typhoid bacilli persist longer in bacterially pure waters than in waters containing large numbers of bacteria, and Houston has shown that raw river water infected with 'uncultivated' typhoid bacilli from a case of typhoid bacilluria cleared itself of 99·99 per cent. of typhoid bacilli after a week, and apparently the organism had completely disappeared in ten days (*cf.* pp. 202, 211, 225).

When they are present, infusoria and crustacean organisms devour typhoid bacilli. Whipple and Mayer found that in water kept under anaërobic conditions (atmosphere of hydrogen) typhoid bacilli quickly died, although under similar conditions it may thrive in nutrient media. Houston found that between 0° and 18° C., the higher the temperature the quicker was the disappearance

of typhoid bacilli in water. Provided it be sterile, organically polluted water such as sterile sewage does not clear itself any quicker than a pure sterile water. Hewlett says that in aerated water (CO_2), *B. typhosus* does not survive a fortnight.

Very different periods of survival have been found by different workers, even a year in unsterilised tap water having been insufficient for it to die out.

Shellfish.—Oysters, winkles, mussels, cockles, etc., from contaminated water may be a source of infection (see p. 207). Klein has shown that oysters readily take up *B. typhosus*, but clear themselves of the ingested typhoid bacilli if they are kept in clean water which is frequently changed. The process is slower if they are kept in a 'dry' state—*i.e.*, out of the sea-water. Oysters from a polluted locality clear themselves of the ingested bacilli to a less extent and at a slower rate, even if kept in clean sea-water, than oysters clean at starting. Oysters from a polluted locality containing a large number of the *B. coli* very rapidly clear themselves of this microbe, whether kept in or out of the water. This shows that *B. coli* is foreign to the oyster, and is rapidly destroyed by it. When, therefore, it is present in the oyster, it must have been derived from the surroundings. However largely infected with typhoid bacilli, the oysters remain in all parts of normal aspect. Cockles and mussels similarly take up the typhoid bacillus, but clear themselves much more slowly, particularly in the case of cockles, than do oysters.

The Board of Agriculture and Fisheries in their Report for 1912 suggested a period of quarantine in pure water for shellfish before sale.

Dust and Air.—Infection through the air for any distance, though possible, is unlikely. In India, where excreta are buried in the ground, and dust-storms are frequent, water-supplies and food are sometimes contaminated by fæcal-loaded débris. Where latrines are employed, as in military camps, it has been found that the spot from which an epidemic started was that nearest to, and directly to leeward of, the filth trenches; and the direction in which it spread was always down the wind.

Flies.—After feeding on excreta, and other infected material, bacilli adhere to the legs and wings of these

insects, and should any be swallowed they may pass through the fly unhurt. Living bacilli have been found in or on the bodies of flies twenty-three days after infection. Dutton infected two healthy persons by exposing them to bed-bugs that had previously bitten a typhoid case.

Vegetables.—Watercress grown in sewage-polluted streams, and vegetables from sewage farms, are proved to have been the means for the conveyance of the disease. Creel ascertained that plants from seed sown in infected soil carried typhoid bacilli on their leaves for over thirty days when shade was provided. In full sunlight the typhoid bacilli lived ten days at the outside.

Soil.—Whereas in peat the bacillus dies within twenty-four hours, in moist earth it may exist for two or three months, but shows no tendency to increase in numbers.

Milk.—Through being handled by infected persons, through the use of polluted water for rinsing out utensils or for purposes of dilution, typhoid bacilli can obtain access to milk, and it is probable that multiplication may take place. The organism will survive for at least twenty days, even after the milk has turned sour and curdled. Butter made from infected milk retains the power of infection for a time, and it is possible that the organism may exist in curd cheeses. A series of cases on the round of a specific milkman is the usual clue to the origin of a milk epidemic. There is no evidence to warrant a suggestion that cows may suffer from and transmit the disease.

Filters.—A filter such as the domestic carbon type, which only arrests a portion of the micro-organisms, if contaminated by the passage of an infected water, will continue to infect water passing through it, even if this be pure.

Contact.—Direct infection of a healthy person by a typhoid case is not uncommon. The 'typhoid carrier' (*vide infra*) is especially dangerous on account of his condition being probably unrecognised.

Pathogenesis.—The bacillus can nearly always be found in the blood during the fever period, the number being greatest during the early stages of the fever. The number decreases with the temperature, and disappears when the latter reaches normal. Inflammation and

necrosis of the Peyer's patches is set up, forming ulcers, which may become so deep as to lead to perforation. The bacillus may rarely be detected in the fæces and urine during the early stages of the disease, but appears in the former after the eighth or ninth day, and from urine enormous numbers can sometimes be found after the third week. The organism may be found in the spleen, sweat, mesenteric glands, liver, kidneys, bone-marrow, and in some cases of pneumo-typhoid in the sputum. From its presence in the kidneys, rose spots, and urine, Wright and Semple consider the disease to be a septicæmia. The dorsi-lumbar region is not a very uncommon site for the late activity of the bacillus ('typhoid spine'). Such complications as cystitis (from infection by urine) and inflammations of the gall-bladder are common. Typhoid bacilli persist in the gall-bladder for a long time and are suggested to be a frequent nucleus of gallstone. Typhoid pleurisy is rare and typhoid meningitis rarer still. Osteomyelitis may develop six or seven years after recovery from typhoid (Jordan). Injected into animals, the typhoid bacillus produces a general septicæmia. Animals, with the exception of chimpanzees and young suckling rabbits, are immune to the bacillus administered *per os*. In young rabbits a febrile affection is produced, with diarrhœa and inflammation and ulceration of the lymphoid (Peyer's) patches and solitary glands. The organism is most readily demonstrated in, and isolated from, the spleen of a cadaver, in which it is found in the form of small aggregates or colonies.

The view that the first lesion necessarily takes place in the ileum is giving way before the idea that an entrance into the blood-stream is effected through the tonsil or other alimentary lymphoid area.

Typhoid Carriers.—After cessation of the fever, the bacillus disappears from the fæces and urine fairly soon as a rule, but a certain proportion (Semple gives it as 11·6 per cent.) of cases are infectious for more than six weeks after infection (chronic carriers). It is estimated that three or four persons in every thousand are carriers. Cammidge records a case where the bacilli were still being excreted in the fæces ten years after recovery from the disease. The gall-bladder and the urinary passages are the sources from which the fæces and urine are infected.

Mild types of the disease are as likely to give rise to bacilli carriers as the severest forms. Typhoid carriers may also be recruited from those who have not passed through an attack of the disease in a clinically recognisable form and from those who have been in contact with typhoid cases. The handling of food by such persons is a great source of danger, and outbreaks of the disease have been definitely traced to them.

Serum-Therapy.—Chantemesse grows the typhoid bacillus in a maceration of spleen, bone-marrow, and defibrinated human blood. This, on the injection of an animal, produces a serum which, Wright suggests, perhaps owes its value to the presence of toxins and not of antibodies. Macfadyen and Hewlett, by injecting horses with the cell-juice of typhoid bacilli, prepared by grinding the bacilli in the presence of liquid air, obtained a serum of promise.

Anti-Typhoid Vaccine (Wright).—Typhoid bacilli (the virulence being kept by intraperitoneal passages through guinea-pigs) are grown in nutrient broth in flasks, at 37° C., for a maximum period of thirty-six hours, and not that of ten to fourteen days as formerly. A temperature of 60° C. as originally employed for killing the bacilli seriously affected the keeping property of the vaccine. Now, after the contents of several flasks have been mixed (to get a uniform product), the culture is killed by exposure to 53° C. for an hour. Portions for testing sterility by both aërobic and anaërobic methods are removed and the bulk is allowed to cool, when $\frac{1}{4}$ per cent. of lysol is added. (Addition of lysol to the *hot* vaccine destroys the immunising properties.) This killed culture forms the vaccine, which is standardised by estimating the content of bacilli (see p. 24). A dose of 500 million is injected subcutaneously, and is followed by a febrile reaction, which soon passes off. To obtain more complete protection a second injection of twice the original dose may be given after an interval of ten to fourteen days. The success of this vaccine is remarkable both as a prophylactic and as a curative measure, and an expectation that it will clear the organism from carriers appears reasonable. In the Expeditionary Force in France the ratio of attacks is fourteen times and of deaths forty-two times greater among the uninoculated men. The vaccine loses its

properties by degrees, and should never be used if it is older than three months.

Antityphoid vaccine does not protect against the paratyphoids (*q.v.*). Inoculation has proved to be quite a harmless procedure, the great majority of men inoculated experiencing nothing more than 'inoculation fever.' Very occasionally it starts pyogenic organisms, already lurking in the body, into activity.

Bacteriological Diagnosis—Blood Culture.—Two to five c.c. of blood are withdrawn from a superficial vein with a sterile syringe, and small flasks of broth (25 to 50 c.c.) are inseminated, each with 1 to 2 c.c. of the blood, and incubated. This method serves to differentiate cases of paratyphoid from true typhoid by the differences in the bacilli isolated. It is useful for diagnosis during the first week.

Widal's Reaction (see p. 19).—The lobe of an ear is washed with ether or some other suitable liquid. With a straight, spear-pointed surgical needle a prick is made into the lobe and the blood collected in a capillary tube, preferably sterile. An ordinary vaccine tube does very well. The blood should form a continuous column in the tube. The ends of the tube should be cautiously sealed in the flame, dry end first, care being taken that the blood is so far away from the ends as not to become heated. It is better if the tube can be centrifuged, so as to obtain a clear serum; if this cannot be done, it will be found that after a few hours, on breaking off the ends of the tube, a string of clot and some serum can be blown out on to a glass slide, and the serum with some corpuscles collected in another capillary tube, or in the diluting pipette to be described immediately. If the tube has been centrifuged, it is broken off at the junction of serum and corpuscles, and the clear serum blown out.

A hæmocytometer pipette may be used for diluting, or a pipette may be made by drawing out a piece of glass tubing in the blowpipe flame. A little of the serum is drawn up into it, a mark made on the glass at the upper limit of the serum, and the serum blown out into a watch glass. Then any desired number of similar volumes of diluting fluid (0·7 per cent. salt solution) are added to the serum, and the whole mixed.

Alternatively, Delépine's method may be adopted:

Fifteen drops of broth or salt solution are successively taken up in a loop of platinum wire, and placed on a glass slide; close to them is placed one drop of the serum, and the sixteen drops are then thoroughly mixed. A small drop of this diluted serum is mixed on a cover-glass with an equal-sized drop of a broth culture of the typhoid bacillus. This gives a total dilution of the serum of rather over 1 in 30.

The culture should preferably be from a specimen of low virulence, but it is not an essential point; some cultures agglutinate much better than others. It is also better (but again not essential, if free from clumps) for the culture not to be over twenty-four hours old. If no broth culture is available, an agar culture rubbed up in broth or salt solution will do as well, but should be filtered through filter-paper to remove clumps before use. Dead broth cultures (killed by heating to 65° C. for an hour) may also be used. A control hanging-drop preparation of the culture should always be made, and examined to see that no clumps of bacilli are present in it.

The mixed drop of diluted serum and of culture is examined as a hanging-drop (p. 47) and should have very little depth, as the 'clumps' have a tendency to sink and get beyond the focal distance of the lens. At first the bacilli may be active, but if the case is one of enteric fever they soon slow down or stop, then gradually groups of two or three form; these groups soon aggregate until nearly all are collected into various crowds or 'clumps,' with very few isolated bacilli left. If the reaction is complete within thirty minutes, the case is certainly one of enteric fever. Generally it is not complete, and there may be groups of only ten or twenty, but the occurrence of grouping and loss of movement are in themselves decisive.

The dilution of the serum is necessary, because the serum of normal individuals will often, undiluted, evince an agglutinative power, but not, so far as experience goes, in a total dilution of more than 1 in 16. More than one dilution should always be examined, and it is advisable that one should be high, such as 1 to 80 or 1 to 100. The lower dilutions may conveniently be 1 in 30 and 1 in 50.

The time-limit is necessary since many normal sera will act, even when diluted, if left an indefinite time; with a dilution of 1 in 30 to 1 in 50 an hour should be

sufficient. Time and dilutions should always be recorded. A positive reaction with a 1 in 80 dilution would in almost all cases indicate the bacillus to be either specific for the serum or very nearly so. It sometimes happens that agglutination is obtained with a certain dilution but not with a lower one, *e.g.*, the 1 in 50 may be positive and one of 1 in 16 may be negative. Such 'zone reactions' are very rare.

The agglutination method is very satisfactory. It is not obtained until about the seventh day of fever, is rarely intermittent, and a negative result should not be accepted unless repeated three or four times at intervals of a few days; it is rarely absent throughout the course of the disease, and in such cases the disease is frequently severe. The reaction persists for years after an attack, and, therefore, before applying the test a previous attack should, if possible, be excluded. Inoculation with Wright's vaccine also causes the blood of the inoculated to acquire agglutinative properties. Pyrexia leads to a diminution or disappearance of inoculation agglutinins, and Tidy says that a positive Widal reaction after the sixth day of pyrexia is as definite a proof of *B. typhosus* infection in an inoculated as in an uninoculated man. Delépine isolated a strain of typhoid bacillus, designated as 7120, which clumped with the serum of an active case of typhoid, but not with the serum of an artificially inoculated person. The blood in paratyphoid infections may sometimes agglutinate the typhoid bacillus, but usually only in low dilution. Agglutometers for performing the test by macroscopical observation with dead typhoid bacilli are sold.

Ophthalmo-Diagnosis.—Chantemesse applies typhoid toxins to the conjunctiva—a reaction analogous to Calmette's tuberculin conjunctival reaction.

Cutaneous Reaction.—Deehan applies a drop of fluid containing the standardised toxin to the skin, and then makes a slight abrasion with a lancet under the drop. The reaction, which does not cause the patients any discomfort, is supposed to be specific.

Cultivation from Fæces and Urine.—Surface-plate cultures (p. 237) on Conradi-Drigalski agar, MacConkey's lactose bile-salt agar or malachite-green agar are made with the material, which should be as fresh as possible.

The fæces are first diluted by emulsifying 1 or 2 grammes of fæcal matter with about 700 c.c. of sterile normal salt solution; when the emulsion has stood for about one hour, so as to allow the coarser particles to subside, a small quantity of the supernatant fluid is pipetted off for plating. It is necessary to use three large Petri dishes for each case; about four drops of the fluid emulsion is transferred to plate 1, and carefully spread over the surface with a glass rod spreader bent at right angles. The same spreader, but without being reinfected, is then carefully rubbed in succession over the surface of plates 2 and 3; all three plates are then incubated at a temperature of 37° C. for twenty-four hours.

Colonies resembling typhoid or paratyphoid are worked up.

To inhibit *B. coli*, some workers make a primary inoculation of fæces into peptone water every 5 c.c. of which contains 0·2 c.c. of a 1 in 10,000 solution of brilliant green, incubate for one or two days and then plate out. The simple plating is, however, quite satisfactory.

With urine it is only necessary to spread a small quantity, varying from a few drops to 1 c.c. or thereabouts, over the surface of a plate containing one of the media mentioned, and then to proceed as above.

The Dysentery Bacillus.

The several varieties of dysentery are due to different etiological agents. In one form—the tropical or amœbic—an amœba is believed to be the causative organism (see p. 174); in a second form, the so-called epidemic or bacillary, a bacillus (*B. dysenteriæ*) is the infecting agent. The paratyphoid bacilli and *B. pyocyaneus* may also cause a dysenteric condition, and possibly other organisms—*e.g.*, the *B. coli* and *Proteus vulgaris*—and certain parasites—*e.g.*, the bilharzia—produce a pseudo-dysentery.

As many as fifteen types of dysentery bacilli are said to exist, the Shiga-Kruse and the Flexner groups being best known.

Morphology.—The dysentery bacilli form no spores and are Gram-negative. They are generally said to be non-motile, and most workers have failed to demonstrate flagella.

SOME IMPORTANT MEMBERS OF THE COLON-TYPHOID GROUP.

	Motility.	*Gelatin.*	*Glucose.*	*Lactose.*	*Maltose.*	*Saccharose.*	*Mannite.*	*Dulcite.*	*Litmus Milk.*			*Glucose Neutral Red Broth.*	*Indole.*	*Voges-Proskauer.*
									1st Day.	3rd Day.	12th Day.			
B. typhosus	+	–	A	–	A	–	A	–	A	A	A or Alk	–	–	–
B. fæcalis alkaligenes ..	+	–	–	–	–	–	–	–	Alk	Alk	Alk	–	–	–
B. paratyphosus A ..	+	–	AG	–	AG	–	AG	AG	A	A	A	–	–	–
B. paratyphosus B ..	+	–	AG	–	AG	–	AG	AG	A	Alk	Alk	F	–	–
B. enteritidis (Gärtner)	+	–	AG	–	AG	–	AG	AG	A	Alk	Alk	F	+	–
B. dysenteriæ (Shiga)	–	–	A	–	A	–	–	–	A	Alk	Alk	–	+	–
B. dysenteriæ (Flexner)	–	–	A	–	A	–	A	–	A	Alk	Alk	–	+	–
B. Morgan No. 1 ..	+	–	AG	–		–	–	–	–	–	Alk	–	+	–
B. suipestifer	+	–	AG	–	AG	–	AG	A	A		Alk			–
B. coli	+	–	AG	AG	AG	±	AG	AG	AC	AC	AC	F	+	–
B. coli anaerogenes ..	–	–	A	A			A		A	A	A		+	
B. cloacæ	+	L	AG	AG	AG	AG	AG	–	AC	AC		F	+	+
B. lactis aerogenes ..	–	–	AG	AG	AG	AG	AG	–	AC	AC	AC	F	+	+
B. acidi lactici ..	–	–	AG	AG	AG	–	AG	–	AC	AC			+	–

A=Acid production. Alk=Alkali production. C=Curd production. G=Gas production.
+ =Presence of motility, production of indole, or positive Voges-Proskauer reaction.
L=Liquefaction. F=Fluorescence.

Culture.—The bacilli are aërobic and facultatively anaërobic. The growths on surface agar and gelatin are much like those of typhoid; there is no liquefaction of gelatin. In broth and peptone-water there is a general turbidity without pellicle; indole may or may not be formed. On potato the growth is usually thin and colourless, sometimes yellowish or brownish. In milk it grows well without clotting, the reaction being first acid, and generally after a few days becoming alkaline. It ferments glucose with the production of acid, but no gas; lactose is not attacked, and neutral red is unchanged.

The varieties are identified by agglutination reactions and action on mannitol. The Flexner type ferments mannitol with production of acid, but no gas, while the Shiga-Kruse bacillus has no action on this polyhydric alcohol. Some varieties ferment maltose and saccharose as well.

The thermal death-point is about 60° C. Soluble thermostable toxins are formed in alkaline media, and Todd has been able to produce an antitoxin by treating an animal with the toxin.

Agglutinating Reaction.—Provided the type of organism causing the disease be employed, the blood-serum of patients with bacillary dysentery gives an agglutination reaction, sometimes in high dilutions. Whereas agglutination can be obtained on the second day in some cases, in others it is not found till the twelfth day.

Pathogenesis.—The liver abscesses generally found in amœbic dysentery do not occur in the bacillary disease, and the organism is not found in the urine or blood, but it is abundant in the bowel discharges. The incubation period, after the bacilli have been swallowed, is from one to two days.

The organism is met with in the so-called ulcerative colitis, or 'asylums dysentery,' and in a considerable proportion of cases of epidemic (summer) diarrhœa of infants.

Direct or indirect contact with infected bowel discharges is recognised as a principal means of infection. After apparent recovery the convalescent may still excrete the bacilli. Polluted water has been held responsible for infection, but Forster has shown that the Shiga bacillus has but a short life outside the body, and

Buchanan (*Medical Press*, June 30, 1909) is of opinion that water is not so often to blame as is generally imagined.

MacConkey's lactose agar or Conradi-Drigalski agar may conveniently be used for isolation of the bacilli from fæces. On the former white colonies, and on the latter small clear blue colonies, are worked up.

CHAPTER VIII

THE DIPHTHERIA BACILLUS

Morphology.—The Klebs-Löffler bacillus (*B. diphtheriæ*) is a delicate, slender, non-motile, and non-sporing organism, having rounded ends. Its length is variable, long, medium, and short varieties being described, the usual length seen being 3 μ to 5 μ. Both in the membrane and in cultures a remarkable parallelism in arrangement is seen, and club-shaped forms are frequent.

It is not killed by drying; dust containing the bacillus will retain its virulence for months under certain conditions. The organism is aërobic and facultatively anaërobic, and its thermal death-point is 58° C.

Staining.—When stained with Löffler's alkaline methylene blue, the staining is frequently irregular, darker and lighter stained portions alternating—the so-called 'segmentation'; sometimes the poles are deeply stained, appearing as dark dots at the ends—'polar staining'; and occasionally there is a change of tint to a pinkish here and there—'metachromatism.'

Wesbrook divides the bacillus into three types: The 'solid' type stains uniformly; the 'barred' form shows intervening segments, which either stain but slightly or not at all; and the granular type (which predominates in clinically characteristic affections) has deeply staining granules. The bacillus is Gram-positive, and as confirmatory stains Neisser's or Pugh's stains are generally used.

Neisser's diagnostic stain: A preparation for about thirty seconds is treated with the following solution: One gramme of methylene blue dissolved in 20 c.c. of alcohol, and mixed with 950 c.c. of distilled water and 50 c.c. of glacial acetic acid. It is rinsed in water, treated

with Gram's iodine solution, and then counter-stained in Bismarck brown (see p. 50) for about a minute, washed, dried, and mounted.

The Klebs-Löffler bacillus treated by this method appears as a delicate rod, stained pale brown, and containing two inky-blue dots, one at each end. Sometimes a dot is also seen in the centre. Most other organisms simply stain brown, without any dots. (In the original method the two staining solutions were alone employed; Tanner introduced the use of Gram's iodine. Both solutions should be filtered before use.) In some instances it is possible to obtain characteristic stained preparations from the cultures after five or six hours' incubation, long before there is any visible growth.

Pugh's stain consists of 1 gramme of toluidine blue dissolved in 20 c.c. of absolute alcohol, and made up to a litre with distilled water. Fifty c.c. of glacial acetic acid are added, and the stain filtered. Cover-glass preparations are stained for five minutes, and then washed in water. This stain shows up the granules very distinctly and saves the necessity for a double stain.

Cultural Characters.—On gelatin the growth is slow, without liquefaction. On agar at blood-heat growth is more rapid; on potato, unless first moistened with beef broth, the growth is scarcely perceptible. On blood-serum and glycerin agar growth is rapid, but Löffler's medium (p. 39) is most used, as the growth which appears as a cream-coloured streak along the line of inoculation is so rapid as to allow the bacillus generally to outstrip other organisms that may have been present in the throat, and is visible in twelve hours. A number of small isolated dots near to, but not touching, the actual streak is suggestive of the organism.

The bacillus exhibits hæmolytic property, a yellowish area encircling a colony on blood-agar. Hofmann and xerosis bacilli are said to be devoid of this power. Milk is turned acid without coagulation. Acid, but no gas, is produced in glucose and lactose media.

In peptone-water, after a few days, an indole reaction can be obtained with sulphuric acid; this is not due to indole, but to skatole-carboxylic acid (Hewlett).

The bacillus (and some staphylococci) grows red with a bluish-pink tint diffusing through the medium on a

glucose neutral red sheep-blood serum containing 1 per cent. potassium sulphocyanate (Myer Coplans). *B. Hofmanni* grows yellow.

Bacteriological Diagnosis.—The diphtheria bacillus can occasionally be identified by direct examination of a teased-up fragment of membrane, or in expert hands it may be recognised in a smear made from a swabbing. But generally a cultivation is necessary. The throat may be touched with a platinum wire, and successive streaks made along the surface of a sloped tube of Löffler's serum. Or an 'outfit,' consisting of a sterile cotton-wool swab in a sterile tube, may be used. The swab is rubbed over the suspected portion of the throat, replaced in the tube, and sent to the laboratory. The use of antiseptic gargles or tablets shortly before swabbing must not be allowed, nor should swabbing be performed soon after a meal, since particles of food containing bacteria may render the examination more difficult. The early morning is a most suitable time for routine swabbings. In contacts and convalescents, where no obviously diseased patches are to be seen, the swab should be rubbed on both tonsils. The swab is rubbed over the surfaces of two serum-tubes in turn thoroughly, so that all parts of the swab come in contact with the serum, but lightly, so as to avoid ploughing up the surface, and the tubes are incubated at blood-heat for twelve to twenty-four hours. Cover-glass preparations are then made from the most likely colonies. If no growth is visible, a scraping of the whole surface should be taken, and should a microscopical examination of this preparation be negative, the tubes should be incubated for a further twenty-four hours.

Millard, while regarding the 'long granular' organism as the *B. diphtheriæ par excellence*, expects solid stained bacilli to show granules if further incubated. For diagnostic purposes the barred forms are less satisfactory, as *B. coryzæ segmentosus*, commonly found in the nose in a common cold, *B. xerosis* (*vide infra*), and *B. auris* and *B. ceruminis*, found in the ear, are very similar.

Diphtheroids.—In many healthy throats or throats diseased but not with diphtheria, are to be found bacilli that closely resemble the Klebs-Löffler bacillus in every particular except that of virulence, and it can hardly be

doubted that they are really diphtheria bacilli which have lost their virulence. The Hofmann bacillus ('pseudo-diphtheria bacillus') differs from the Klebs-Löffler bacillus in several particulars. It is a shorter, plumper, somewhat spindle-shaped rod, staining pretty evenly, only exceptionally presenting involution forms, and not giving the Neisser staining reaction. Like the true diphtheria bacillus, it stains well by Gram, and has the same parallelism in arrangement. Its most marked distinguishing feature is that it produces alkali and not acid in milk and glucose media. It is virulent to many small birds, but not to guinea-pigs, and Salter has claimed that, though not forming *toxin*, it does form *toxoids*, which are capable of combining with diphtheria antitoxin, but his results have not been confirmed by Hewlett nor by Petrie. Some have believed that the Hofmann bacillus is a modified and non-virulent Klebs-Löffler bacillus. Most observers have failed to transform the one bacillus into the other, though success has been claimed in a few instances. The Hofmann bacillus seems to replace the diphtheria bacillus in the fauces during convalescence, and this has suggested the transformation of the latter into the former. But as Cobbett pointed out, diphtheria bacilli being soon found in the acute stage, Hofmann bacilli might pass unnoticed, but would be found when scarcity or absence of Klebs-Löffler bacilli necessitated a longer microscopical examination. Present opinion inclines to regard the Hofmann bacillus as a species distinct from the Klebs-Löffler bacillus. In view of this uncertainty, cases of 'angina,' in which the Hofmann bacillus only can be isolated, are often treated as infective. Van Riemsdyk (*Lancet*, 1915, ii. 766) says that true diphtheria bacilli inhabit the throat for choice and not the nose; pseudo-diphtheria bacilli, contrariwise, are most often found in the nose and rarely in the throat. The Hofmann bacillus has been found in the nasal mucus of as many as 71 per cent. of dwellers in large towns.

Ford Robertson believes the specific organism of general paralysis to be a diphtheroid organism occurring in the cerebro-spinal fluid (*B. paralyticans*), and reports remissions in patients by the use of a vaccine.

The xerosis bacillus, an organism met with on the

conjunctiva, is extremely like the diphtheria bacillus in morphology and staining reaction, but it grows more slowly, and the cultures are thinner, drier, and more granular than those of the diphtheria bacillus, and it is non-virulent.

These organisms can be differentiated by fermentation reactions. Buchanan obtained excellent results by coagulating ox-serum in an equal quantity of water, filtering the mixture, adding 1 per cent. of glucose to one-half and 1 per cent. of saccharose to the other half, and 'tubing,' neutral red being used as an indicator. In twenty-four hours a marked acid reaction was produced in the glucose tube by *B. diphtheriæ*, and in both tubes by *B. xerosis*, while no change was produced in either tube by the bacillus of Hofmann.

Hiss's serum-water medium is also used for the fermentation reactions: Serum 1 part, water 3 parts, with 1 per cent. of a carbohydrate or glycerin, tinged with litmus.

The virulence of the bacillus varies considerably. The average amount of a forty-eight hours' broth culture required to kill a 250 to 300 gramme guinea-pig within one to two days is 0·5 to 1·0 c.c.

Channels of Infection.—Diphtheria is particularly a disease of the young between the ages of two and ten; the mortality is greatest at ages below five, and during the last quarter, and is lowest during the summer months. Its distribution is world-wide, but it is most prevalent in cold and temperate climes. The 'school influence' is an undoubted factor in disseminating the disease, there being a decrease in the number of cases during holidays. The disease may be acquired by direct infection, as in kissing, or by less direct means. The organism retains its virulence for a long time in particles of dried membrane, sputum, and discharges. As a rule, the bacilli disappear from the throat within three or four weeks of the beginning of the attack, but they often persist for longer periods. Millard found bacilli present thirty days after admission in 25 per cent. of his cases, and a case of Hewlett's repeatedly gave the organism for twenty-two weeks after the commencement of convalescence. A case should not be pronounced as being free from infection until at least two, preferably three, examinations have shown that the bacilli are no longer present.

While non-virulent diphtheria-like bacilli are frequently present in milk and its products, virulent Klebs-Löffler bacilli have several times been isolated from milk. Klein stated that cows inoculated in the shoulder with the bacillus are attacked with an eruptive disease of the udder, and that the diphtheria bacillus could be isolated from their milk. Abbott and Ritter, repeating Klein's experiments, failed to find the bacilli in the milk. Dean and Todd investigated a small outbreak of diphtheria, in which the Klebs-Löffler bacillus was isolated from the milk and from an eruption on the udders of two cows supplying it. They seem to have proved, however, that the eruptive disease was not due to the diphtheria bacillus, but was an eruption which had become infected with the bacillus, and they suggest that both the lesions on the cows and the milk had become infected from some outside source, possibly the milker. Milk epidemics are generally attributed to a human rather than a bovine origin. Experiments show that the bacillus may survive for some weeks in spring waters of little organic impurity, although during this time the virulence is gradually attenuated. If, however, at any time previous to its ultimate disappearance the organism be transplanted into a suitable culture medium, it can reacquire its full initial virulence. There is, however, no well-authenticated instance in which water has been proved to be the source of infection.

Faulty sanitary conditions may also assist in the spread of this disease by preparing the throat for the bacillus, and may in this way apparently give rise to cases which would never have arisen had it not been for the existence of such conditions.

It is not unusual for an epidemic of diphtheria to be preceded by a prevalence of 'sore throat,' which seems to gather in intensity till cases arise of undoubtedly true diphtheria.

Pathogenesis.—The incubation period varies from two to seven days, but is usually from about two to four days. The mortality is about 0·20 per cent. of the total death-rate.

Mucous surfaces are most prone to infection. The pharynx is most generally the area attacked, but infection of the larynx (membranous croup) and of the nose

(membranous rhinitis) are common. The middle ear and the mucous surfaces of the genitals may also be sites of infection. Traumatic diphtheria may arise through contact of an abraded surface with the organism. Conjunctival infections have been caused by patients coughing or sneezing in the eyes of attendants. In the skin an eruption indistinguishable from eczema is produced. The tonsil plays an important part in the defence of the body and in pre-antitoxin days the mildness and low mortality rate of tonsillar, as compared with other diphtherias, was attributed to this defence.

Occasionally the organism may produce a septicæmia, but generally only the toxins circulate, the organism remaining more or less localised at the seat of infection (toxæmia).

In a typical case a white wash-leather-like membranous coating, consisting of a fibrinous exudation, is present, and on detachment leaves a bleeding patch. Absorption of the toxin produces lesions in the heart, nerves, and kidneys, and paralytic sequelæ may follow recovery from an attack. Hewlett says that paralytic sequelæ are not found when infection of a non-diphtheritic nature is concerned.

Kanthack and Stephens found that in fatal cases diphtheria bacilli can, almost without exception, be detected in the lungs, generally in the cervical and bronchial glands and spleen, and sometimes in the kidney.

The term 'hæmorrhagic diphtheria' is applied to those cases in which, in addition to other signs of malignancy, hæmorrhages appear in the skin at an early stage of the disease, with or without hæmorrhages from the mucous membranes (Rolleston, *Medical Press*, 1909, 390). The mortality is over 80 per cent., reaction to antitoxin is delayed, and all the cases which recover suffer from extensive paralysis.

A person may have the bacillus in the throat without contracting the disease. It may be found in 20 per cent. or more of contacts. Once lodged deep in the lacunæ of the tonsil, the bacillus remains there and the patient becomes a 'carrier.' Pybus records a case that had three attacks of diphtheria in as many years and none after removal of the tonsils. A. G. Macdonald says that the length of carrier-life of the bacillus appears to have no

effect upon its virulence, since the organism has been proved to be virulent after four and eight months in the ear and nose.

The guinea-pig, rabbit, dog, cat, horse, and cow, are all susceptible to infection with the diphtheria bacillus. Rats and mice are refractory. A disease of cats appears to be identical with human diphtheria. They may also harbour diphtheria bacilli without symptoms of the disease.

Although both are susceptible to infection with the Klebs-Löffler bacillus, the diseases known respectively as diphtheria of poultry and calves (p. 121) are of a different nature, and non-communicable to man.

Mixed Infections.—In the false membrane the Klebs-Löffler bacillus is often associated with such organisms as *Streptococcus pyogenes*, staphylococci, and pneumococci. In such cases a high temperature and fœtid throat may be expected, and probably the pathologic process is more serious. W. J. Wilson says that staphylococci may be antagonistic to diphtheria bacilli.

Diphtheria Toxin and Antitoxin.—The nature of the extracellular toxin is not known, but it probably consists of protein. When injected into an animal in doses, sublethal at first and gradually increasing, an antitoxin is formed.

In addition to the toxin, a diphtheria broth culture contains other substances called *toxones* and *toxoids* that combine with antitoxin. Toxoids are non-toxic derivatives of toxin. Toxones are primary secretory bodies that produce induration, necrosis, and paralysis.

Preparation of the Toxin.—A virulent culture (grown in slightly alkaline beef broth at 37° C., with free access of air, for a week) is filtered through a Pasteur-Chamberland filter. The toxicity of the filtrate should be such that not more than 0·01 c.c. will kill a 250-gramme guinea-pig in forty-eight hours.

Immunisation of the Horse.—A small dose of toxin is injected into the withers, together with a dose of antitoxin. A slight swelling appears, and after a few days subsides, when the operation is repeated, using a larger quantity of toxin. A swelling again appears; when this has in turn subsided, further injections are made, till it is possible to inject 500 c.c. of toxin without injury

to the animal. When sufficiently immune, a sterile cannula is inserted in the jugular vein, and the blood drawn off into sterile bottles. The bottles are placed in an ice chamber to allow the clot to separate. The clear serum is separated for use. A small quantity of trikresol is added. For use in hot climates the serum may be evaporated to dryness *in vacuo*, and then forms amber-coloured scales or granules, which, for use, may be dissolved in five to ten parts of sterile water (one part of dry serum = about ten parts of the fluid serum).

There is gradual diminution of antitoxic power in the serum yielded by horses, even though they continue to receive toxin, so after some months fresh animals have to be employed.

The preparation of a horse to give serum of very high antitoxic value by the above method involves treatment extending to six months or more. Cartwright Wood grows the diphtheria bacillus in ordinary peptone broth containing serum for three or four weeks at 37° C., and, after filtration, heats for an hour at 65° C. It is thereby claimed that powerful antitoxic serum can be produced in a short time.

During immunisation a small quantity of blood is withdrawn from time to time, and its antitoxic power tested.

Standardisation of the Serum.—The serum is tested against a certain amount of the toxin, the result being recorded in units. The methods of Behring and of Roux were formerly employed for this purpose.

According to Behring's standard, a serum that contains *one normal antitoxin unit per c.c.* is of such a strength that $\frac{1}{10}$ of a c.c. completely neutralises the action of ten lethal doses of toxin.

The lethal dose of toxin is the amount required to kill a given weight of guinea-pig. Of this toxin, ten times the amount required to kill a guinea-pig of about 250 grammes weight is injected, together with the antitoxin to be tested. By noting the absence or presence of local reaction and the increase or loss of weight, it is stated that an opinion may often be formed after twenty-four hours, but that after forty-eight hours a decision can always be given. When the toxin is completely neutralised, as it should be, the animal should not only live, but there should be no trace of local reaction (œdematous swelling). This

swelling may not be apparent in the first twenty-four hours, but a rapid fall in weight will at that time frequently indicate its probable occurrence within the next twenty-four hours. In this connection it should be borne in mind that guinea-pigs, taken from stock and put into small cages, usually rise in weight when not injured by the action of the toxin.

Roux's method defines the proportion of serum in relation to the weight of the animal which would protect a guinea-pig against a lethal dose of toxin.

Ehrlich's method of standardisation, now universally adopted, eliminates errors due to toxoids and toxones. A standard antitoxin is employed, as this is more stable than toxin if dried and stored *in vacuo*. For use, this is diluted so that one unit is contained in 1 c.c. The laboratory toxin is then standardised with this standard antitoxin, and the exact amount of the toxin is ascertained, which, when mixed with one unit of antitoxin, just suffices to cause the death on the fourth or fifth day of a 250-gramme guinea-pig. This amount of the toxin is termed the L+ dose (L = *limes* = boundary—*i.e.*, between life and death, the neutral point—L+ meaning that there is one lethal guinea-pig dose of the toxin left unneutralised by the unit of antitoxin). The L+ dose of the laboratory toxin having been ascertained, this amount of the toxin is mixed with varying amounts of the antitoxic serum to be tested, and each mixture is injected into a 250-gramme guinea-pig. The amount of the serum which just suffices to completely protect the guinea-pig from the toxic effects of the L+ dose of toxin is then known to contain one unit of antitoxin. The standardised toxin is preserved by the addition of toluol, and is kept in a cool, dark place; but even then its toxicity gradually diminishes, and it has to be restandardised every three to six weeks.

As Sudmersen and Glenny found that the active immunity of the doe guinea-pig is transferred passively to her offspring, animals from parents used previously in the test cannot be used.

The unit of antitoxin corresponds to 105 to 115 minimal lethal doses of a toxin for the guinea-pig, or, roughly, to 100 minimal lethal doses.

The most suitable place for injection of antitoxic

serum is the subcutaneous tissue of the flank. The dose given varies from 2,000 to 4,000 units up to 30,000 or more. A child may require as much as an adult.

Injections of serum are often followed by the appearance of various rashes, sometimes erythematous, at other times urticarial, and in a few cases not unlike the rash of scarlet fever or of measles. These rashes usually come on in from seven to ten days after the injection; sometimes the rash is accompanied by more or less pyrexia, and in a small number of cases by pains, and even effusion into some of the joints. Such rashes may appear after injection of normal horse-serum (see Serum Disease, p. 26).

Diphtheritic paralysis seems to occur more frequently than formerly, the explanation being that more cases, and especially severe ones, recover. For cases treated on the first day of the disease the mortality is nil, but rises with delay in treatment, until in cases not treated until after the fourth day the mortality is nearly as great as in pre-antitoxin times. In many cases the injection of the serum is followed by a speedy reduction in the severity of the symptoms, and a rapid separation of the membrane in cases where it was causing obstruction of the air-passages, thus diminishing the number of cases which would otherwise require tracheotomy.

Prophylactic Use of Diphtheria Antitoxin.—To protect contacts the prophylactic dose should be about 500 units. The immunity so obtained does not last longer than three weeks.

Vincent's Angina.

This affection of the throat often closely simulates diphtheria, but the diphtheria bacillus is absent. In the lesions Vincent describes the presence of two symbiotic organisms—one a bacillus, with pointed ends 6-8 μ to 10-12 μ in length and 1-1·5 μ in diameter, sometimes motile, not staining by Gram, and cultivable anaërobically on the ordinary media with the addition of human serum (*B. fusiformis*). With this is usually associated a long, delicate, motile spirillum (*Spirochæta Vincenti*), which has not been cultivated, and which is supposed to be the fusiform bacillus in another stage.

Diphtheria of Birds.

'Pigeon diphtheria' appears to be generally due to an organism resembling the bacillus of hæmorrhagic septicæmia, but the 1907 epidemic in wood-pigeons and some other epidemics were presumably due to Löffler's *B. diphtheriæ columbarium*, a short, non-motile organism, which is Gram-negative and forms no spores.

Diphtheritic roup of poultry is supposed to be due to a protozoon. Macfadyen and Hewlett isolated a Klebs-Löffler-like organism from the throats of healthy birds, but the organism was non-virulent to guinea-pigs. Jordan states that an organism usually present in roup differs essentially from the Klebs-Löffler organism; diphtheria antitoxin is without effect upon the progress of roup. A tough, yellow, false membrane is found on the conjunctivæ and the mucous membranes of the mouth, pharynx, and breathing passages of the birds, and, apart from the question of transmissibility of the disease to man, the affected birds are emaciated to a degree that renders the flesh unfit for food.

Calf Diphtheria (malignant or ulcerative stomatitis) is said by Hewlett to be produced by an anaërobic streptothrix, while Stockman attributes it to Bang's necrosis bacillus.

CHAPTER IX

THE BACILLI OF HÆMORRHAGIC SEPTICÆMIA

This class of organisms comprises the infecting agents of bubonic plague, rabbit septicæmia, swine plague, fowl cholera, and septic pleuro-pneumonia of cattle. These affections are characterised by the presence of hæmorrhagic areas under the skin and throughout the internal organs. The organisms themselves all show bipolar staining (*i.e.*, the ends of the organisms stain deeply, whereas the central portion hardly stains at all). The bacilli are Gram-negative, are short and non-motile, and do not form spores.

Bacillus Pestis.

Morphology.—In the body it occurs as a short, almost ovoid, rod, measuring on the average about 2·3 μ by

1·7 μ; but longer forms are seen, measuring 5 μ. As convalescence approaches, round and ovoid involution forms, totally unlike the bacillus, appear. In cultivation the young bacilli are so short as to be almost coccoid or slightly oval, but in older cultures rod, thread, and involution forms occur. In broth culture the organism forms chains (streptobacillus).

In a film made from an agar culture the bacilli are swollen and yeast-like, but it is questioned whether a capsule exists.

Staining Reactions.— *B. pestis* does not stain by Gram's method, but with ordinary dyes shows marked bipolar staining.

Cultural Characters.—*B. pestis* is an aërobe and a facultative anaërobe. Growth is slow at 18° to 20° C., rapid at 37° C., but 30° C. is the optimum temperature.

In broth a characteristic flocculent, wavy deposit is formed, which settles to the bottom, leaving a clear medium above.

In broth containing a little butter or coco-nut oil, and kept absolutely at rest, flocculent, tapering masses of growth depend from the droplets of oil floating on the surface (Haffkine's stalactite growth).

On the surface of gelatin it forms a thin, whitish, punctate growth, which is confined to the inoculation streak; the medium is not liquefied. On the surface of agar and of serum it forms a thick, cream-coloured, very sticky growth.

On agar containing 2·5 to 3·5 per cent. of common salt pear-shaped and spherical involution forms are so common that Hankin recommends this salt agar as a diagnostic medium. It grows in milk without coagulation. On potato little or no growth takes place. It grows well in bile-salt media. While the virulence of some strains is retained in cultures for a long while, that of other races quickly diminishes.

Resistance.—The organism is very easily killed by disinfectants. The thermal death-point appears to be between 58° and 70° C. While cold has little effect, complete desiccation kills the bacillus. In sterilised water the bacillus has remained alive for fifteen days at room-temperature.

Pathogenesis.—Three types of the disease are common —bubonic, septicæmic, and pneumonic. The bacillus is found in the buboes (sometimes with streptococci and staphylococci) in the bubonic form, in the blood-stained (' rusty ') sputum in the pneumonic form, and in the blood in the septicæmic form. It is also found in the blood in the other forms on the approach of death. The period of incubation appears to be usually from three to six days, but may extend to nine days.

Pathogenesis for Lower Animals.—The injection of cultures into mice, guinea-pigs, and rats, produces plague symptoms, and the animals die in two to seven days. A mere scratch with a needle dipped in an emulsion of a recent culture of plague will generally kill a white mouse of ordinary size in one to three days. Rats and mice can be infected *per os*. The bacilli are found in the spleen and lymphatic glands of inoculated animals but are not very numerous in the blood.

Calves and poultry may contract the disease in a chronic form, as the result of feeding on plague-infected offal (Simpson). Plague also attacks cats, dogs, ferrets, bats, squirrels, pigs, hares, rabbits, and monkeys.

Transmission.—The plague bacillus probably obtains entrance through wounds in the skin, or through the mucous membrane of the respiratory or (more rarely) alimentary tracts. The sputa of patients with the pneumonic disease, when discharged in droplets during coughing, etc., probably constitutes the main way in which disease spreads in these cases.

The lower animals often convey the disease. An epizoötic among rats is almost constantly seen before the disease becomes epidemic among men, and ground squirrels have been held responsible for epidemics in California. Rats may become infected by ingestion of carcasses of men and animals dead of the disease. When infection is experimentally produced in this way mesenteric buboes are most frequent. But Jordan says that an examination of 5,000 naturally-infected rats showed no instance of mesenteric bubo, cervical buboes being most often found. Different species of rats vary greatly in regard to their susceptibility to the disease. The white rat is the most susceptible, the brown ship rat or brown dock rat coming next, then the black rat and a

Norwegian species, and finally, the least susceptible of all, our own common sewer rat.* In the Bombay district, a new outbreak first attacks *Mus decumanus* (the grey sewer rat), passing to *Mus rattus* (the black house rat), and then on to man. The disease is largely spread by fleas. On the death of an animal the fleas desert it for a living host. The common rat-flea found in the tropics (*Xenopsylla cheopis*) readily attacks man, and laboratory experiments show that, provided a healthy animal can be protected against the fleas of one that is plague-stricken, the former is not likely to develop the disease, in spite of the proximity of the respective cages. (*X. cheopis* has only been found once on English rats.)

Sambon insists that in epidemic plague transmission from man to man is more frequent than transmission from rat to man. He believes that during a true epidemic the rat strain of *B. pestis* is replaced in many cases by a human strain, and the rat fleas are replaced by the fleas of man, and by those of the cat and dog, which attack man as well. Jordan states that cervical buboes are most common when flea infection has occurred.

Serum Treatment.—A horse is inoculated intravenously with a suspension of dead bacilli weekly for three months. During the next three months living organisms are given. Various reports have been given of the efficacy of serum treatment.

Anti-Plague Vaccine.—The 'Haffkine prophylactic' is prepared by growing a virulent bacillus in nutrient broth containing butter-fat. After a week the stalactite growth (see p. 122) is detached by shaking, and the broth reinoculated with the organism. By the time the medium has been treated thus four or five times development becomes slow and scanty. The culture is sterilised by heating to 65° C. for one hour, and 0·5 per cent. of carbolic acid added. The dose employed is about 2·5 c.c., which is injected subcutaneously. A second injection, given a week after the first, increases the immunity. By the use of the Haffkine prophylactic the incidence of the disease is much reduced, and among the vaccinated who contract the disease the mortality is much less than among the unvaccinated.

* 'The Bacteriology and Etiology of Oriental Plague' (Klein).

Bacillus Pseudo-Tuberculosis Rodentium.

A disease occurring spontaneously in guinea-pigs and rabbits, accompanied by wasting, and progressing to a fatal issue in about three weeks, is caused by the *B. pseudo-tuberculosis* of Pfeiffer. This bacillus grows readily and rapidly, forms a creamy growth on agar, and a whitish growth on gelatin without liquefaction, not unlike that of the colon bacillus. It also grows well in bile-salt media (MacConkey). It is not acid-fast, and does not stain by Gram. It has been met with in milk and sewage. MacConkey has shown its fermentation attributes to be similar to those of *B. pestis*. In the Suffolk plague epidemic of 1910, a few rats whose appearances were suggestive of plague, were found to be infected with *B. pseudo-tuberculosis*.

Swine Plague.

In the lung lesions of pigs affected with swine plague (contagious pneumonia of swine), an organism very similar to the bacillus of chicken cholera is found, but is not regarded as the cause of the disease. Stockman is of opinion that this disease comprises cases of swine fever with pneumonia as a complication, and confirms McFadyean's opinion that these organisms are normal inhabitants of the mouth and air-passages in pigs, which, owing to the weakened powers of resistance, have been able to invade and multiply in the lung tissue.

Chicken Cholera.

This disease as a rule runs a very rapid course, prominent symptoms being profuse diarrhœa, drooping wings, ruffled feathers, and somnolency. It is said to be sometimes introduced into this country through foreign maize. In an epidemic investigated by the authors, a fresh consignment of maize had certainly been fed to the birds shortly before the outbreak. The specific bacillus is a small oval bacillus, which exhibits polar staining so markedly as to look like a diplococcus in stained preparations. The organism can be readily found in the blood, but Stockman points out that the presence of such an organism is, from his experience, insufficient to prove cause of death, as bacilli of a similar type, which may in

no way be connected with death, are not infrequently present in the heart-blood of fowls. There seems little doubt that the organism is identical with the bacillus of rabbit septicæmia, and probably with the so-called bacillus of swine plague. It differs from the similar bacilli isolated from the following diseases in that the latter are not communicable to the fowl: 'grouse disease,' epizoötic pneumo-pericarditis in turkeys (McFadyean), and cholera in ducks. It also differs slightly from *B. septicus agrigenus*, an organism isolated by Nicolaier from manured soil, and from an organism found by Klein in fowl enteritis.

B. choleræ gallinarum has been described as an infecting agent in some cases of gunshot wounds in the head at Alexandria (Bartlett).

The bacilli of fowl cholera and its allies show very little, if any, multiplication in bile-salt media (MacConkey).

The Committee of Inquiry on Grouse Disease decided that lesions thought to be due to acute and infective pneumonia in grouse were in many cases nothing more than normal post-mortem changes and therefore dismissed the bacteria isolated from further consideration.

The undermentioned organisms differ from those previously described in this chapter in retaining the stain by Gram's method.

The Bacillus of Swine Erysipelas.

Many cases of swine erysipelas (commonly known as nettle-rash) amount to little more than a passing indisposition. There is a rise of temperature, more or less shivering and loss of appetite, followed by a patchy red eruption on the skin about the base of the ears, thighs, and body. In severe cases there is vomiting and great prostration, the pigs stagger about, breathe rapidly, and perhaps die in about forty-eight hours. The specific organism is a non-motile, Gram-positive bacillus, with rounded ends, about 2 μ long. It is found in the spleen, bone-marrow, and lymphatic glands.

A vaccine is used, together with an anti-serum for prophylaxis, producing an active immunity lasting about a year. This is largely a seasonal disease, and animals should be immunised before the season of prevalence—not later than May. Swine erysipelas is said to be communicable to man, though rarely.

Bacillus Murisepticus.

The bacillus found in mouse septicæmia is similar to if not identical with the bacillus of swine erysipelas. In the tissues it is an extremely minute bacillus, 1 μ in length, but in cultures filamentous forms occur. It stains well by Gram's method. It is aërobic and facultatively anaërobic. On surface agar it forms minute delicate colonies, or a thin, delicate, greyish film, not unlike that of the streptococcus; in stab gelatin it grows well without liquefaction, forming a well-defined but delicate cloud-like growth radiating from the stab.

CHAPTER X

MICRO-ORGANISMS OF SUPPURATION AND SEPTIC DISEASES

THE action of organisms is not an indispensable condition for the formation of pus. Abscesses may be produced by the injection of turpentine, sodium cinnamate, and other substances. Such an abscess intentionally produced (Fixation Abscess) is used by French surgeons for relief of certain bacterial infections, toxæmias, and even for cases of metallic poisoning (Brelet, *Medical Press*, 1915, i., 440). Pus may also be produced by the introduction into the tissues of sterilised bacteria of several kinds, with or without the soluble products of their growth, so that the exciting cause may be either the intracellular contents of the organisms, or, possibly, the mechanical effect combined with the positive 'chemiotaxis' that most bacteria and their products exhibit to the leucocytes.

Aseptic pus (sterile pus) is, however, rarely encountered, and pus formation (suppuration) is almost always due to microbic agency. An organism capable of producing pus is termed 'pyogenic.' An abscess is a local and well-defined collection of pus, and the activities of these so-called septic organisms may produce boils, carbuncles, erysipelas, cellulitis, septicæmia, sapræmia, and pyæmia (for definitions, see p. 14). According to the presence of factors other than the organisms, different

manifestations may be brought about by the same species. By the injection of a broth culture of *Staphylococcus pyogenes aureus* into the blood-stream of a rabbit a septicæmia alone is produced, except perhaps in the kidneys, where abscesses may be formed. If, however, before injection the culture is rubbed up with finely-ground potato, abscesses will be produced in the heart and a pyæmic condition be induced, owing to the inability of the potato particles, with the bacteria adherent thereto, to pass through the capillaries. The washing out of a localised abscess causing a sapræmia results in an improvement of the condition of the patient, owing to the removal of organisms producing the offending toxins.

In addition to the organisms dealt with in this chapter the following are also pyogenic: the tubercle, typhoid, colon, and glanders bacilli, the pneumococcus, the *Actinomyces*, and certain of the *Blastomycetes* and *Hyphomycetes* (*q.v.*).

Staphylococcus Pyogenes Aureus.

Morphology.—This organism is a spherical coccus, about 0·75 μ to 1 μ in diameter, and occurs as a diplococcus, or, more commonly, in grape cluster-like masses. It is non-motile, forms no spores, and is Gram-positive.

Cultural Characters.—The coccus is an aërobe and a facultative anaërobe. It grows well at room-temperature and at blood-heat. The virulence of cultures persists for many months. In broth a general turbidity forms within eighteen hours. Gelatin begins to liquefy as soon as there is any visible growth, liquefaction occurring in stab culture all along the stab, an orange-yellow sediment being produced. On agar and blood-serum a thick streak develops, which is at first pale, but later becomes golden-yellow; exposure to diffused daylight favours chromogenesis.

Resistance.—The thermal death-point is 58° C. (Sternberg), provided the organism is in a moist condition; if desiccated, much greater heat is required. It also seems that certain of the cocci in a culture are more resistant than the majority to destruction by heat and antiseptics.

Habitat.—The surface of the body appears to be the normal habitat; it has been found in dust, earth, and water, but its presence in these is probably accidental.

Pathogenesis.—Different strains exhibit considerable variation in virulence. Injected subcutaneously, it forms a local abscess; into the circulation, a septicæmia; into the peritoneum, a purulent peritonitis; and rubbed into the skin, local inflammation, with small pustules (impetigo). Eczema is now generally regarded as being due to the irritative action of the *chemical products* of *S. pyogenes aureus*. The organism has been found in ulcerative endocarditis, furunculosis, osteomyelitis, empyema, boils, carbuncles, and abscesses, in acne pustules, and occasionally in septicæmia and pyæmia.

Little or no toxin is formed by *S. pyogenes aureus*, and attempts to prepare an antiserum have proved unsuccessful. Vaccine treatment of staphylococcal infections is, however, quite popular.

Other Pathogenic Staphylococci.

Staphylococcus pyogenes albus and *S. pyogenes citreus* are not to be distinguished from the *aureus* variety, except by the colours of the growths on agar or potato. The former produces white and the latter lemon-yellow growths. Andrewes and Gordon regard these three organisms as a single species, owing to their ability to produce intermediate varieties of colour on cultivation. The *albus* is said to be frequently found in styes. A feebly virulent variety of the *S. pyogenes albus* is frequently present on the skin—*S. epidermidis albus*. Welch finds it to be so deeply buried in the epidermis as to render it difficult to destroy by means of disinfectants. It is the most frequent cause of stitch abscess. While *S. pyogenes aureus* ferments mannite, with production of acid, this organism has no action.

Staphylococcus cereus albus forms a greyish-white, wax-like growth, and *S. cereus flavus* a wax-like growth, first white, and then yellow, on gelatin. Neither liquefies gelatin. These organisms are specially met with in localised inflammatory conditions, and negative results follow inoculation experiments.

Micrococcus salivarius, occurring in the saliva, and a micrococcus found in scurf from the scalp, give white growths on agar, and are non-pathogenic for animals.

Pathogenic Tetracocci.

Micrococcus tetragenus occurs in phthisical cavities, and sometimes in the pus of acute and chronic abscesses. The organism occurs in pairs or fours, often seen surrounded with an ill-defined capsule. It is Gram-positive, slowly develops on gelatin as a thick white growth without liquefaction, and on injection into white mice produces general septicæmia.

Micrococcus catarrhalis is present in pairs or tetrads (often within the polymorphonuclear leucocytes) in the discharges of the 'influenza cold' and other forms of nasal catarrh and bronchitis. It is sometimes concerned in producing pyorrhœa and other buccal abscesses. It grows at 22° C. It is Gram-negative, and produces opaque colonies of so tough consistence on serum agar that they often come away intact on a platinum loop.

Sarcina ventriculi occurs in the stomach, particularly in cases of dilated stomach.

Bacillus Pyocyaneus.

The *B. pyocyaneus* is a small bacillus found in blue and green pus, very actively motile, non-sporing, giving a creamy growth on agar, to which it imparts a greenish fluorescence and rapidly liquefying gelatin, the fluid being similarly coloured. It may not produce blue pigment for some days, and perhaps not at all until it has been passed through animals (Lartigau). It does not stain by Gram. The pigment can be extracted with chloroform, and consists of two pigmented bodies—*pyocyanin* and *pyoxanthose*. The organism occurs in various conditions, accompanied by debility, wasting, and diarrhœa—the so-called marasmus—and occasionally its presence in water has caused dysentery. It has also been responsible for septicæmia. There are probably several varieties of the organism, varying in virulence and pigment production, of which the *B. fluorescens liquefaciens*, an organism common in water, may be one. A body of the nature of a ferment, 'pyocyanase,' when extracted from cultures, is used for the prophylaxis and cure of anthrax and diphtheria.

The Acne Bacillus.

Unna's *B. acnes* occurs in many conditions of the skin, such as acne vulgaris. It is Gram-positive and resembles Hofmann's bacillus. Sabouraud regards it as the cause of seborrhœa, but proof of this is thought to be wanting by Cooke and Dold. The organism, which is a facultative aërobe, is most easily grown anaërobically on glucose agar. Raised greyish-white opaque colonies appear in three to five days. Sabouraud's original medium consisted of agar, 15 grammes; peptone, 20 grammes; glycerin, 20 grammes; distilled water, 1 litre; and concentrated acetic acid, 5 drops. Fleming's medium (*Lancet*, April 10, 1909) consists of oleic acid (1 to 5 per cent.) in nutrient agar. Western (*British Journ. Dermat.*, January, 1910) and others are of opinion that infection of the sebaceous material with the acne bacillus is a secondary event, and leads, by the irritation caused by its presence, to proliferation of the adjacent epithelium and formation of the comedo. Autogenous acne bacillus vaccine is recommended for treatment, but this has apparently failed in seborrhœa (*Practitioner*, 1910, 526). Cases with much induration are most frequently due to a mixed infection of the acne bacillus and *Staphylococcus albus*, sometimes with *S. aureus* or *citreus* as well.

Streptococcus Pyogenes.

Morphology.—In pus a chain may contain up to fifteen elements, but much longer ones of thirty or forty elements are met with in broth cultures, of which the individual cocci may vary very much in size, both large and small cocci being found in one chain. It also sometimes happens that a new chain starts away from one of the cocci in a chain, thus producing branching. The variation in the size of the cocci is also noticed in cultures on other media. The organism is Gram-positive.

Cultural Characters.—The organism grows well in the presence or absence of oxygen. When grown in broth at 37° C., the medium becomes turbid in twenty-four hours; after three or four days multiplication ceases, owing to the production of an inhibitory metabolic substance, but living organisms have been found after ninety days.

Growth on gelatin is slow and without liquefaction. The colonies are generally very small and discrete, while on agar at 37° C. the colonies are also small and seldom coalesce with neighbouring colonies that almost touch. This tendency of streptococcal colonies to remain small is of great assistance in picking them out from plates containing a variety of bacteria. In stab or shake culture in gelatin the colonies appear as small whitish spheres. In milk, acid is produced without a clot. It ferments lactose, saccharose, and salicin. Growth on blood agar shows it to be strongly hæmolytic. The thermal death-point lies between 52° and 54° C. (Sternberg).

Pathogenesis.—Streptococci of the *pyogenes* type are more frequently found to be concerned in pathogenic processes than are those of the short chain varieties. It is found in ulcerative endocarditis, occasionally in osteomyelitis and frequently in pyæmia, mammary abscess, cellulitis, lymphangitis, and other suppurative conditions. *S. pyogenes* is generally supposed to be identical with *S. erysipelatis*, an organism found at the periphery of the zone of redness in the lymph channels of the skin in cases of erysipelas. Some doubt exists whether the infective agent in puerperal fever is *S. pyogenes* or an allied organism (see *S. puerperalis*, p. 133). There appears to be little doubt that several species of streptococci have been described under the name of *S. pyogenes*.

Serum Treatment.—The virulence of a culture of *S. pyogenes* is 'exalted' by passage through a series of rabbits. A culture medium of 1 part of human or ass's serum with 2 or 3 parts of broth is then inoculated, and a horse is immunised, first with killed and then with living cultures. A serum prepared with one variety of streptococcus may not immunise against another, so the horse is inoculated with several strains of streptococci ('polyvalent' serum). This serum is antimicrobic. Vaccines of killed cultures of streptococci are also used.

Coley's Fluid.—An attack of erysipelas supervening upon a malignant growth has sometimes caused the disappearance of the latter, and sterilised cultures of the *S. pyogenes* have been used in the treatment of inoperable tumours with varying success. Coley grows *S. pyogenes* and *B. prodigiosus* together for two or three weeks, and

the cultures are sterilised by heating to 65° C. Injection is followed by a marked temperature reaction. The treatment appears to be more successful in sarcoma than in carcinoma.

Streptococcus Puerperalis.

While puerperal fever may be caused by *B. coli* and *Staphylococcus pyogenes aureus*, in most cases it is a streptococcus infection, and this is popularly supposed to be *S. pyogenes*. While this may be the case, a streptococcus found in the uterine discharge and in any secondary pus, pleuritic fluid, or sputum by Mackey and Furneau Jordan is considered by them to be probably the most frequent cause. *S. puerperalis* grows freely upon agar, producing opaque colonies which are much larger than any other streptococci, producing chains of moderate length. It produces acid and clot in milk, acid in lactose, glucose, saccharose, and salicin, but no change in raffinose, mannite, and inulin. Furneau Jordan suggests that this streptococcus, like *S. fœcalis*, is present in the contents of the bowel, and that the puerperal woman is very susceptible to its action.

Diplococcus Rheumaticus.

Poynton and Paine's organism is usually a diplococcus measuring 0·5 μ in diameter, but forms masses on solid, and chains in liquid, media. It stains feebly by Gram, grows slowly on gelatin without liquefaction, and forms in broth a flocculent deposit with clear supernatant fluid. On agar it forms minute, white, discrete, slightly opaque colonies, on potato hardly grows, and in litmus-milk forms much acid and a firm clot. It is found in the arthritic and valvular lesions in rheumatic fever, and on inoculation into rabbits may produce arthritis and endocarditis. Poynton and Paine's organism has been found in affected tonsils in the tonsillitis preceding rheumatic fever (Pybus).

Varieties of Streptococci.

Attempts at classification have hitherto proved unsatisfactory. Lingelsheim attempted a distinction between the long-chain (*S. longus*) and short-chain (*S. brevis*) streptococci, the former being considered more virulent

than the latter. But virulent short-chain streptococci are sometimes met with, and it is possible to transform one variety into the other by culture. Penfold describes the variations obtained as 'haphazard.' Strains that correspond to hæmolytic streptococci have been converted into typical pneumococci and *vice versa*.

Gordon divides the streptococci into four groups: (i.) *S. longus* (from the mouth). Very long and comparatively straight chains. Broth remains clear with flocculent deposit. Acid but no curd in milk. (ii.) *S. medius* includes most pyogenic streptococci, which as a rule form fair-sized curling chains; corresponds to Lingelsheim's *longus* type. Broth acquires a flocculent deposit but supernatant liquid remains clear. Milk becomes slightly acid without curd. (iii.) *S. brevis*. Short chains. Broth uniformly turbid. Slight acid and usually curd in milk. Includes pneumococcus. (iv.) *S. scarlatinæ* or *conglomeratus*. Masses of chains. Deposit in broth but upper liquid keeps clear. Acid and curd in milk. Later, Gordon introduced fermentation tests as a basis of differentiation and using these Andrewes and Horder distinguish (1) *S. pyogenes*, already described. (2) *S. salivarius* (found in saliva), a *brevis* type that clots milk, is not hæmolytic nor pathogenic for mice. (3) *S. anginosus* (inflamed fauces, scarlatinal throats, and rheumatism), a *longus* type, with no action on salicin, is hæmolytic and pathogenic for mice. (4) *S. fæcalis*, a fæcal organism sometimes found in cystitis, meningitis, and pus. A *brevis* type that clots milk, is not hæmolytic nor pathogenic for mice, the only class to ferment mannitol. Perhaps the *Diplococcus rheumaticus* is identical with this organism. (5) The pneumococcus (*q.v.*). (6) *S. equinus* (from horse dung), a *brevis* type, not clotting milk and the only class that does not ferment lactose.

Too much stress must not be placed on these differences, experience showing the characters mentioned are anything but absolute.

Salivary streptococci do not usually ferment salicin, and Savage thinks the discovery of salicin-fermenting streptococci in throats during an epidemic of sore throat would point to a milk infection.

Streptococcus mastitidis, found in streptococcic inflammation of the mammæ of cows (mastitis), and *S. an-*

ginosus, found in inflamed and scarlatiniform throats, are said to be indistinguishable morphologically and culturally (Local Government Board Reports, 1907-08, 'Garget in Cows'). *S. mastitidis* only produces a local abscess in animals, however, while *S. anginosus* produces general symptoms and death. The former produces mastitis in goats, while the latter does not.

Jordan thinks most of the streptococci in milk are probably descended from saprophytic, not from pathogenic, ancestors (see pp. 221, 222).

Wright (*Lancet*, October 30, 1915) describes the most frequent organism in wounds received in action as a streptococcus that, in film preparations of pus, nearly always shows up as a diplococcus. As obtained from agar and broth cultures, the elements of the diplococcus are lancet-shaped and the pair form an angle resembling a circumflex accent. In broth, a few short chains are also formed. Colonies on agar show up very faintly grey-green and when planted closely tend to run together. Growth is much more rapid than with ordinary *S. pyogenes*, luxuriant cultures being obtained at 37° C. on broth or agar in four or five hours. In normal serum, and on agar when transplanted in blood, it grows out. Wright finds this organism to correspond to the *enterococcus* of the French authors, and he also regards it as the ordinary streptococcus of fæces (see p. 265).

Mutch says that in diabetes the duodenum is infested by the *S. brevis* and that the only other condition in which this abounds in the duodenum is rheumatoid arthritis. Rosenow considers it reasonable to suppose that in man gastric ulcer may be caused by streptococci. Houston describes a case in which phthisis was simulated by a streptococcic infection and in a case of duodenal ulcer he found in the contents of the stomach streptococci and staphylococci, from which a vaccine was made with promising results. Streptococcic infections occur in measles leading to septicæmia, which may prove fatal in the middle or end of the second week. Streptococci are frequent in alveolar abscesses and infected root canals: Gilmer and Moody found many varieties, including a hæmolytic streptococcus with a wide zone of hæmolysis, *S. mucosus* (see p. 141) and *S. viridans*. (*S. viridans* resembles *S. salivarius*, but grows green on blood-agar.)

Strangles, an equine disease, is supposed to be due to streptococci, and they have been found in scarlet fever (p. 195), variola (p. 200), and other diseases.

The Gonococcus.

Morphology.—The gonococcus is a small organism measuring about 0·7 μ by 0·5 μ, tending to be somewhat like a coffee-bean in shape, usually grouped in pairs, the flattened sides of the two organisms being adjacent, occasionally single or in tetrads. It is killed in ten minutes at 60° C.

Cultural Characters.—The gonococcus is aërobic. Not growing on ordinary media, it can be cultivated on Wertheim's medium, a mixture of equal parts of nutrient agar and human blood-serum, blood-smeared agar, or the medium of Christmas—rabbit's blood-serum coagulated by heat. A simple method of cultivation is to deposit drops of blood obtained with aseptic precautions from the finger on the surface of an agar plate, then to add a drop of gonorrhœal pus, smear over the plate, and incubate at blood-heat. A pure culture of the gonococcus assumes a raised appearance, similar to a mulberry, and is of a greyish-white colour. It is necessary to sub-culture every few days, or the vitality is lost.

Whitehouse uses ordinary agar, with the addition of human blood-serum and a few drops of human urine (*Practitioner*, 1910, 489).

Pathogenesis.—The human urethra is generally the site of attack, producing an inflammation, which may be followed by posterior urethritis and stricture. In gonorrhœa the pus usually contains gonococci in pure culture during the first few days, but later on staphylococci and streptococci will often be found. After the acute stage of gonorrhœa has passed, and there is no longer any considerable flow of pus, the gleet that follows still contains the gonococcus.

After the discharge has ceased, an examination of the centrifuged deposit from the urine may reveal the organism in large numbers. The organism may persist in the genitals for years after apparent recovery, and the patient still be capable of infecting others. In the female the infection often spreads to the Fallopian tubes, ovaries, and peritoneum. Gonorrhœal ophthalmia of the

new-born is due to maternal infection (see Stephenson's 'Ophthalmia Neonatorum'). In the male infection may produce epididymitis and prostatitis. The circulatory system may be invaded and produce arthritis (see Murrell, *Medical Press*, 1910, 87) or endocarditis. Infection may also be transferred by the use of infected towels, sponges, etc. Entrance of the pus into the eye may result in its loss unless properly treated.

Christmas, by growing the gonococcus in a medium containing fluid rabbit's serum, obtained a feeble toxin, with which he immunised rabbits, the serum of which was feebly antitoxic. Vaccines are sometimes used.

Microscopical Examination of Pus.—In the female an examination is best made directly after a menstrual period, as in some cases, especially in chronic and sub-acute conditions, the gonococci are then, and then only, to be found. Films are prepared from the cervix uteri, Bartholin's ducts, and Skene's tubules in the female, or from the discharge as it exudes from the urethra in the male. The films should be fixed by immersion in alcohol and ether (equal parts) for fifteen minutes. Some films are stained with Löffler's methylene blue, and the others by Gram's method. In using the latter method for this organism it is of the utmost importance that the technique be most carefully and religiously observed. Bismarck brown is a suitable counter-stain (Neisser's *b.* stain, acting for two minutes.) Identification of the organism is only permissible when *all* the following characters are shown. The organism must completely lose its stain when treated by Gram's method, should have the coffee-bean form, and occur in the pus cells. In the early stage of infection the organisms may be found outside the cells. Abundance of other organisms may lead to the gonococci being missed, and in all cases a negative result should be received with great caution.

The characters described will usually suffice to distinguish the gonococcus, but in cases of importance cultures should be made. Organisms similar to the gonococcus are found in the genitals, but these grow on gelatin, with liquefaction of the medium, and on nutrient agar. Some of these urethral diplococci are Gram-positive, and there is no doubt that they are sometimes responsible for the urethritis or whatever form the infection takes

Eyre and Stewart state that the *B. xerosis*, which is frequently present in the healthy and seldom absent from the diseased, genital tract, forms colonies on blood-agar which are indistinguishable from those of the gonococcus to the naked eye.

Seeligman considers pruritus vulvæ to be due to a diplococcus, which resembles the gonococcus in appearance, but is Gram-positive.

The Meningococcus.

Morphology.—In epidemic cerebro-spinal meningitis ('spotted fever') the causative organism is the *Diplococcus intracellularis meningitidis* of Weichselbaum (meningococcus).

While found in the polymorphonuclear leucocytes of or lying free in the cerebro-spinal fluid as a diplococcus, on culture it is a markedly pleomorphic organism, and involution forms appear on media early. Lundie, Thomas, and Fleming (*Lancet*, September 25, 1915) state that associated with the meningococcus in the naso-pharynx and brain, there is nearly always a Gram-positive 'streptococcus,' tending on culture to become Gram-negative and producing involution forms—in the blood they are often Gram-negative. Donaldson (*Lancet*, June 26, 1915) suggests the true cause of cerebro-spinal fever to be a diphtheroid bacillus of which the meningococcus is only a phase. Lundie, Thomas, and Fleming have also described pseudo-diphtheroids and they state the dots do not stain by Neisser like the Klebs-Löffler bacillus. These authors (*loc. cit.*) describe the 'caterpillar,' 'Zeppelin,' and 'bomb' forms also met with.

Like the gonococcus, which it resembles in appearance and arrangement, the meningococcus is Gram-negative. It is very susceptible to cold, and infected swabs or other material intended for culture experiments must be kept as near body-heat as possible, if an interval between sampling and plating-out is unavoidable. Halliday Sutherland (*Lancet*, October 16, 1915) says the meningococcus soon dies at 72° F. and is killed in thirty minutes at 62°.

Culture.—For primary cultures from suspected material, blood-smeared agar may be used, but Wassermann's nutrose ascitic agar ('Nasgar') is the popular medium.

Nasgar is prepared as follows: 6 grammes of nutrose and 90 c.c. of ascitic fluid are added to 210 c.c. of distilled water. This is heated with constant agitation to boiling, when the nutrose dissolves. This solution is added to 600 c.c. of nutrient agar (previously melted) and the mixed liquid is heated in the steam steriliser for half an hour, filtered and tubed and sterilised as for nutrient agar. Cultures are incubated at 37° C. and colonies appear in twenty-four to forty-eight hours. The colonies are characteristic. They are pearl-grey, clear, smooth, and translucent with a firm, regular, oval or round outline. They are larger than the colonies of pneumococci and streptococci which are often found in material from the naso-pharynx. The meningococcus ferments maltose and glucose with the production of acid (distinction from *Micrococcus catarrhalis*) and fails to ferment saccharose. Muir and Ritchie point out that these fermentation reactions are most satisfactorily carried out on solid serum media containing one per cent. of the sugar to be tested.

McIntosh and Bullock (*Lancet*, November 27, 1915) use a medium of nutrient 3 per cent. agar, 3 parts; unheated horse-serum, 1 part. The nutrient agar should be neutral to phenolphthalein and made from beef-broth. These authors mention that bile seems to inhibit growth of the meningococcus.

Agglutination reactions may sometimes be obtained, but negative results are worthless. Morgan (*Lancet*, 1909, ii., 156) found that the serum of patients may agglutinate typhoid bacilli in a dilution of 1 in 50—a phenomenon which would be looked upon as a positive reaction to Widal's test.

A recently suggested diagnostic reaction is performed by adding a drop or two of anti-meningococcus serum to a tube of fresh cerebro-spinal fluid obtained by lumbar puncture and cleared by centrifuging. The tube and a control are incubated at 52° C. for a few hours. If the meningococcus is the cause of the meningitis, a precipitate is said to develop in the tube to which the serum was added, but not in the control.

Channels of Infection and Pathogenesis (see also p. 265). —Cerebro-spinal meningitis is described in the horse, cattle, sheep, and dog, but it is not known if these arise

from the meningococcus, and the probability of the disease in man being derived from animal sources does not appear to be likely. Cerebro-spinal meningitis more often affects country districts than cities (Osler). It arises under cold atmospheric conditions and disappears with the advent of warm weather. Overcrowding, bad sanitation, and privation are cited as predisposing causes.

In the body the meningococcus has primary residence in the naso-pharynx. While it is there the host is a carrier case, often without developing the disease himself. Though carriers are usually free from the meningococcus in two or three weeks, it persists in a small percentage for two or even seven months. Mayer reported a case probably existing two years. Carriers may develop meningitis after two or three weeks. There is considerable variation in the number of carriers among contacts in different epidemics. Among 300 soldiers who were contacts Arkwright only found four carriers. In other epidemics as many as 23 per cent. to 37 per cent. have been reported.

During the stay in the naso-pharynx, whether the host be a healthy carrier or in the incubation stage of the disease, the small drops of secretion expelled during sneezing, coughing, or speaking serve to carry and disseminate the meningococcus. Halliday Sutherland says that infection is carried by currents of warm moving air. Kissing is obviously dangerous. In the developed disease the meningococcus is found in the cerebro-spinal fluid and very often in the blood.

Where a case is definitely attacked, the cerebro-spinal fluid obtained by lumbar puncture shows distinctive features: an increase of pressure, increase in albumin content, and a polymorphonuclear leucocytosis frequently so marked as to render the fluid quite turbid. An examination of the centrifuged deposit shows the meningococci which are often within the leucocytes. If no diplococci are found, recourse must be had to culture, but even then negative results are sometimes obtained when all the other evidence suggests meningococcal activity. Carrier cases are detected by bacteriological examination of the naso-pharyngeal secretion. As several non- or feebly-pathogenic micro-organisms closely resembling the

meningococcus are present in the healthy mouth, contamination of the swab with saliva must, as far as possible, be avoided. West's swab is recommended. The swab from the naso-pharynx is lightly brought in contact with one portion only of ready set nasgar in a Petri. The material from the infected area is spread over the medium by means of a sterile glass spreader, and, without reinfection, this spreader is rubbed over the surface of a second plate. The plates are then incubated at blood-heat. It is imperative that the culture should be made immediately after the swabbing of the naso-pharynx or else the infected swab should be kept warm until it can be used. When the colonies are up, a film is stained by Gram's method, meningococci being Gram-negative. Subcultures are made and incubated at 37° C. and 23° C. respectively. The vast majority of the Gram-negative cocci of the normal mouth grow readily at 23° C., while the meningococcus does not.

Gaskell (*Jour. R.A.M.C.*, September, 1915) says that when the puncture fluid is allowed to stand for twelve to eighteen hours in the blood-heat incubator and the sedimented pus sown on blood-agar slopes, success is more frequent than with generous sowings of fresh fluid.

Organisms that may possibly be mistaken for the meningococcus are—

(1) *Gonococcus.* For practical purposes this may be excluded.

(2) *Micrococcus catarrhalis.* Distinguished by fermentation tests (*vide supra*) and by its growth at 23° C.

(3) *Diplococcus pharyngis siccus.* Grows at 23° C. Colonies are very tough, those of the meningococcus being easily removed and readily emulsified with water.

(4) *Diplococcus mucosus.* Gives slimy colonies. Grows at 23° C.

(5) *Diplococcus crassus.* Gram-positive.

(6) *Chromogenic cocci* are not likely to lead astray, unless the colour does not develop. Under this circumstance *Micrococcus flavus* gives colonies that closely resemble those of the meningococcus.

(7) *Pneumococcus.* Gram-positive.

(8) *Pseudo-meningococcus.*

(9) *Streptococcus mucosus.* Gram-positive; on serum-agar gives a colony as clear as water.

Flexner's curative serum has been used with success (*Lancet*, October 30, 1909), and has considerably diminished the mortality, but it is important to obtain a serum which is polyvalent to as many strains as possible. A serum prepared from one strain may be entirely ineffective when used to combat infection with a meningococcus morphologically and culturally identical.

The judicious use of polyvalent vaccines is said to have met with frequent success. Colebrook (*Medical Press*, May 12, 1915) states that the vaccine did not help to eradicate the meningococcus from the naso-pharynx of carriers with any regularity.

CHAPTER XI

The Diplococcus (Streptococcus) Pneumoniæ.

Morphology.—*D. pneumoniæ* is found in large numbers in the affected lung and in the rusty expectoration of acute croupous or lobar pneumonia, and occasionally in the blood. It is seen usually as a diplococcus, sometimes in chains of four elements. The cocci are oval or lance-head shaped, measure 0·5 μ by 1·0 μ, and are surrounded with marked gelatinous capsules. The organism is Gram-positive. Maynard (*Med. Press*, November 4, 1914) thinks it probable that the pneumococcus can assume the form of a bacillus of diphtheroid type.

Cultural Characters (see also Streptococci, pp. 134, 15).—The pneumococcus is an aërobe and facultative anaërobe. It grows well on blood-serum and glycerin agar at 37° C., but the dewdrop growth is only visible on close examination. On gelatin at room-temperature it does not develop. Under cultivation the capsule is lost, except in media containing blood-serum, and it forms short chains of a few cocci; hence it is probably a streptococcus. It rapidly (five to six days) loses its vitality on agar, but can be preserved alive for a considerable time on agar smeared with blood or in gelatin kept in the *blood-heat incubator*. It does not develop on potato. It grows in milk, with the production of acid, and usually with curdling.

Clinical Examination.—With sputum the microscopical examination and the inoculation of a drop into the peritoneal cavity of a mouse give more reliable results than culture methods, since other species of organisms are frequently present; but with pus and exudations pure cultures can generally be obtained.

Pathogenesis.—*D. pneumoniæ* is pyogenic and may be found in broncho-pneumonias, pleurisy and empyema, endocarditis and pericarditis, meningitis (one-third of the cases), peritonitis, arthritis, osteomyelitis, and conjunctivitis. It can apparently also produce inflammation of the throat, with the formation of a false membrane, and is sometimes met with in this situation in association with the diphtheria bacillus. It frequently occurs in the healthy mouth. As it occurs naturally, its virulence is subject to great variation. In its clinical features pneumonia presents strong resemblances to the specific fevers, and though isolated cases are most common, epidemics do occasionally occur. The diplococcus is very fatal to mice on subcutaneous or intraperitoneal inoculation, less so to rabbits and guinea-pigs, while pigeons and fowls are immune. Antipneumonic serum has not given very satisfactory results.

Vaccine Treatment.—Willcox and Morgan commence treatment with a stock vaccine, while an autogenous vaccine is being prepared by culture: (1) from sputum; (2) from blood; or (3) by aspiration of the pleural cavity or superficial part of the consolidated lung by a small syringe with a fine needle. Vaccine treatment has sometimes been found beneficial.

Resistance.—When protected by an albuminous coating the pneumococcus may retain its vitality for three or four months, and has been found in the dust of a room occupied by pneumonic patients and in the dust of hospital wards.

Mice may play an active part in the dissemination of pneumonia, particularly the epidemic variety (Gamaleia).

The Pneumo-Bacillus of Friedlander.

This organism, in the sputum, occurs as a short rod 1 μ to 2 μ in length, with rounded ends, though longer forms are seen. In the exudations it is encapsuled, and frequently occurs in pairs. It is non-motile and non-

sporing, and is aërobic and facultatively anaërobic. It is Gram-negative.

Cultural Characters.—The pneumo-bacillus grows well on all the ordinary media, both at room-temperature and at blood-heat, but loses its capsule under cultivation. On agar and blood-serum it forms abundant moist, thick, cream-coloured growths. On surface gelatin it forms a whitish spreading layer, and in stab gelatin a nail-shaped growth—the growth being heaped upon the surface like the head of a nail, and tapering from above downwards in the line of the puncture; the gelatin is not liquefied. On potato a well-marked white sticky layer develops. Milk is usually slowly coagulated. There is an abundant growth in broth, with a considerable deposit. It ferments glucose, saccharose, mannite, and lactose energetically, with the formation of gas and acid. Variations in its power of fermenting occur.

It is sometimes met with in the sputum, particularly in bronchitis, and occasionally in association with the *Diplococcus pneumoniæ*. It is also found in stomatitis and rhinitis, ulceration of the cornea, affections of the throat (sometimes with a false membrane), and in broncho-pneumonia.

Mice are susceptible to infection, the guinea-pig is less so, and rabbits are infected with difficulty.

Other Pneumonic Conditions.

There are also pneumonic conditions frequently complicating other diseases, and most frequently seen in young children in the course of measles and whooping-cough, in influenza, in typhoid fever, in plague, and after operations about the mouth and throat ('septic' pneumonia). Although acute croupous pneumonia may complicate these diseases, the pneumonic process is usually of a different type, starting in a number of scattered patches, which, however, may coalesce and so involve large areas.

Micrococcus Melitensis.

The causative agent of Malta or Mediterranean fever is the *M. melitensis*, a small coccus occurring singly, in pairs, or in short chains, with an active Brownian movement. (It is doubtful if it is a true motility, though

flagella have been described by Gordon.) Bacillary forms have been observed in old cultures, and frequently occur in even young cultures. Maynard (*Medical Press*, November 4, 1914) says this bacillary form grows more rapidly and luxuriantly than the typical micrococcus.

M. melitensis is of slow growth, especially in the primary cultures from the spleen; on agar in three to four days it forms small semi-transparent droplets, which later become opaque and yellowish-orange in colour. It develops slowly on gelatin as a limited dirty white streak without liquefaction. It does not stain by Gram's method. Inoculated into monkeys, it produces a febrile condition, with enlarged spleen, simulating the human disease; but it is non-virulent to guinea-pigs and rabbits, except on intracerebral inoculation.

The disease is diagnosed by an agglutination reaction, but dilutions up to 1 in 100 should be prepared, as Hewlett points out that old laboratory strains agglutinate with normal serum in dilution of 1 in 20 or 30. The organism occurs in the blood and milk of goats, and the latter constitutes the main source of infection. Since the prohibition of goat's milk to the garrison at Malta (1906) the disease has practically disappeared. The organism is sometimes found in the urine, and less often in the fæces and milk of patients. It is not apparently a water-borne disease.

CHAPTER XII

The Influenza Bacillus.

Morphology.—Pfeiffer's *B. influenzæ* is found in the sputum and nasal secretion during the febrile period of influenza. It is a very small rod, not exceeding 1·5 μ in length and 0·3 μ in thickness. It has rounded ends, and is generally found in pairs, but on cultivation grows out into strings. It is Gram-negative. When stained with dilute carbol-fuchsin there is a tendency to bipolar staining. Resistance to outside influences is very slight.

Cultural Characters.—The influenza bacillus is a strict aërobe. No growth occurs below 25° C., the optimum

temperature being 37° C. On the surface of blood-smeared agar it forms small transparent colonies, which are perceptible with difficulty. Growth on ordinary agar medium is slight and uncertain, but it grows better in broth containing grape-sugar and glycerin. The organism must be subcultured every eight days on blood-agar, or its vitality will be lost.

Pathogenesis.—The period of incubation is twelve to twenty-four hours. The disease may occur in an uncomplicated form, or may be accompanied or followed by respiratory or gastro-intestinal lesions or neuroses, but no case involving both respiratory and gastro-intestinal lesions has been recorded. The simple form lasts from three to five days, and the complicated from eight to ten, except those affecting the nervous system, when the patient is often months, or even years, in shaking off the effects, and there are a few cases where insanity or paralysis has resulted. It is not uncommon for the same patient to have two attacks in one year, and in each fresh epidemic those who have had the disease once are far more liable to be attacked than those who have previously escaped.

The bacillus varies greatly in virulence and in the type of infection produced. The pneumococcus may be the causative agent in many cases of so-called influenza (Allen). In a small percentage symbiosis of the influenza bacillus with the pneumococcus or *Staphylococcus albus* occurs. Broncho-pneumonia of an epidemic type has been caused by the influenza bacillus. Influenza is transmissible to cats, and is the cause of 'pink eye' in horses.

The Bacillus of Ducrey.

The bacillus of soft sore (Ducrey's bacillus) is found in the ulcers and buboes of soft chancre. It is minute and generally arranged in groups or chains, mostly outside the leucocytes. It does not stain by Gram, and is cultivated on blood-agar, producing small shining grey colonies, or in guinea-pig's blood. It produces the disease on inoculation of the human subject.

The Koch-Weeks Bacillus.

This small bacillus is the cause of an acute contagious conjunctivitis Morphologically it resembles the influenza

bacillus, and growth is difficult except on serum agar (minute transparent colonies) or ascitic fluid glycerin agar. It is not pathogenic for animals.

The Bacillus of Whooping-Cough (Bordet and Gengou).

B. pertussis has much the same characters as the influenza bacillus. It is a strictly aërobic small cocco-bacillus, non-motile, negative to Gram, and staining feebly with methylene and toluidene blues. The best medium is agar, with which has been mixed a large proportion of blood drawn off aseptically. The serum of patients who have recovered from the disease shows specific reactions to this organism. The serum of patients suffering from this disease agglutinates the bacillus sometimes in as much as a sixty-four-fold dilution, and gives the complement deviation method of Bordet-Gengou.

The Glanders Bacillus.

Morphology.—*B. mallei* is a straight or slightly curved rod, 2 μ to 5 μ long, with rounded ends. Stained preparations may show beading or bipolar staining. From the production of long filaments with swollen ends and exhibiting lateral branching, some regard it as belonging to the *Trichomycetes*. It does not form spores, and is non-motile, though an active Brownian movement is present in broth cultures. Its thermal death-point is 55° C. (Löffler).

Cultural Characters.—The glanders bacillus grows slowly on gelatin without liquefaction, and readily on glycerin agar as a creamy layer. On potato the growth, which is apparent in three to four days, at first has the appearance of drops of honey, but later on deepens in colour and becomes thicker, and eventually darker, till it approaches a chocolate colour. The potato itself remains unstained.

Staining Characters.—*B. mallei* is Gram-negative, is not acid-fast and does not readily stain with ordinary dyes. Smears of glanders pus or material are best stained, according to McFadyean, with methylene blue, and then treated with 4 to 5 per cent. acetic acid for a few seconds, which decolorises the nuclear detritus, but still

leaves the bacilli well stained. In sections from the edge of an ulcer, glanders bacilli are few in number and difficult to recognise.

Pathogenesis.—The disease is communicable to man, the horse, mule, ass, sheep, goat, field-mouse, and guinea-pig. Cattle are entirely immune, and white mice and rabbits partially so. Nocard considers the path of entrance to be often through the alimentary canal. This cannot often be the case with human infections. In man glanders occurs generally through the infective discharge from a diseased horse coming into some traumatic injury, and is a very serious affection. In the horse there are a persistent nasal discharge, ulcers on the septum nasi, and usually an enlarged submaxillary gland. In the horse, when the disease affects the skin on the insides of the legs it is popularly known as 'farcy,' but lesions are invariably found in the lungs (McFadyean). The swellings of superficial lymphatics and glands are known as 'Farcy buds.' Suppuration usually follows.

The discharge, either from the nostrils or from ulcers or pus, contains comparatively few bacilli, so that it is not easy to demonstrate the bacillus by staining.

Straus's method of obtaining a pure culture consists in the injection of the suspected discharge into the abdominal cavity of an *adult* male guinea-pig. If *B. mallei* is present the scrotum will be red and shining after three days, and the testicles much enlarged and caseous, and the caseous material will contain the bacillus in pure culture. A similar orchitis may, however, be induced by other organisms, or a fallacious result be obtained through the animal not developing orchitis at all, or else dying from general peritonitis before orchitis has time to develop.

Addison and Hett give the incubation period for man as from a few hours to a year, most generally four to seven days. They also emphasise the necessity for making the injection for Straus's reaction with a culture grown on potato.

Mallein.—The organism is grown in glycerin broth for about six weeks; the culture is sterilised by heat, filtered through porous porcelain; the filtrate when concentrated constitutes mallein. If about 1 c.c. of mallein be injected into a healthy animal, nothing, or

only a slight febrile reaction, occurs, in the horse not exceeding about 102°, the normal being about 100°; but if glandered ever so little, the temperature runs up to 103° or even 106° in eight to sixteen hours. At the seat of inoculation a large swelling appears, and any local lesions, if present, become much enlarged. This swelling is of more importance diagnostically than the rise of temperature.

It was discovered at the Wellcome Research Laboratories that many non-glandered horses, if immunised against other bacterial products such as diphtheria toxin, react to malleïn, but the local swelling rapidly disappears and the rise in temperature persists for a shorter period. With this exception, the malleïn test as a diagnostic agent is practically infallible. It seems to act but feebly as a curative agent, although a few cases of apparent cure after its use have been reported.

The Glanders and Farcy Order, 1894, sec. 17, compels seizure and total destruction of every part of an animal that had glanders at time of death.

Epidemic Abortion in Cattle.

Bang described a very small non-motile, oval or rod-shaped, Gram-negative bacillus, found in the exudate between the fœtal membranes and the uterine mucous membrane, and also in the stomach and the blood of the fœtus. The bacillus was sometimes lying singly, but in many cases was markedly clumped. This clumping is attributed to the agglutinating action of the animals' serum, and is especially noticeable when the bacillus is grown on serum media. Bang described the bacillus as growing in a shake culture from ½ to 2 centimetres below the surface only, but McFadyean's cultures grew best either on or just underneath the surface. The bacillus grew on all the ordinary media, the agar-gelatin serum medium being the most favourable. On agar growth takes ten days or more to appear. On potato the growth closely resembles that of glanders. The organism only grows between 30° and 37° C. The thermal death-point is 60° C.

A Departmental Committee appointed in 1905 issued a Report in 1909, in which Sir John McFadyean and Mr. Stewart Stockman found that, although vaginal

infection may sometimes cause the disease, food containing virulent material is a very important factor in its dissemination. Ewes, goats, bitches, and guinea-pigs can be infected experimentally.

Non-pregnant cattle have been immunised by means of large doses of the living cultures of the abortion bacillus, and treatment of infected cattle by vaccines gave very promising results. Stockman thinks abortions can be reduced from the 30 per cent. that sometimes occur to 6 or 7 per cent. by prophylactic inoculation.

CHAPTER XIII

THE SPIRILLA

The Spirillum of Asiatic Cholera.

Morphology.—*Spirillum choleræ Asiaticæ* (Koch's 'comma' bacillus), as it appears in the excreta, is a curved rod ('vibrio'), 2 μ by 0·3 μ. On cultivation, especially in liquid media, S-shaped and spirillar forms develop. It is actively motile, with a single flagellum at one end only, exceptionally more. No spores are formed. Desiccation and sunlight are rapidly fatal, and its thermal death-point is about 50° C. The organism is Gram-negative, but is readily stained with anilin dyes, especially with dilute carbol-fuchsin.

Cultural Characters.—The spirillum grows readily on most media. An alkaline reaction is essential, development being hindered by small amounts of acid. Aërobic conditions allow a much better growth than anaërobic ones, and its resistance to disinfectants increases as a saprophytic habit develops.

It slowly liquefies gelatin, giving in a stab culture in forty-eight hours a bubble of liquefaction at the top of the stab only. While growing on most media at 22° C., on potato there is no perceptible growth at this temperature. On potato at 37° C. there is a slow, light greyish-brown growth.

The rapid formation of indole is very characteristic of Koch's comma. It gives a creamy growth on agar and

a general turbidity, delicate pellicle and sulphuretted hydrogen in broth cultures.

Bacteriological Diagnosis.—It is frequently possible to report positively at once as to the nature of the disease on the microscopical examination of one of the rice-like flakes, whether from the contents of the ileum or in a living patient from the stool. A minute fragment of one of the flakes is suspended in sterile salt solution (0·7 per cent.) and examined in a hanging drop, when the spirillum of Koch is recognised by its characteristic screw-like movement. If a portion of a flake be crushed carefully between two cover-glasses, which are then drawn apart and stained, the organisms lie with their long axes in the same direction, and present the 'fish-in-stream' appearance. (*Isolated* vibrios may be found in normal dejecta, so no significance can be attached thereto.)

Such appearances are, however, only to be found in perhaps half the cases, and it is generally necessary to perform the following cultural experiments: A flake or two are washed in two or three rinses of sterile salt solution, and then broken up and thoroughly emulsified in a little salt solution. Several gelatin tubes are melted, and inoculated with loopfuls of this suspension, and poured into plates, while at the same time six to twelve flasks containing sterilised Dunham solution are similarly inoculated. (This solution consists of peptone 1 per cent., salt 1 per cent., in distilled water.) The flasks should be conical Erlenmeyer ones of about 120 c.c. capacity, and containing 40 to 50 c.c. of the Dunham's solution. After inoculation the flasks are capped with a loose cap of sterile filter-paper and incubated at 37° C. The gelatin plates are examined after twenty-four hours' incubation at 22° C. The colonies are then macroscopic, and appear microscopically as granular discs, with faintly sinuous margins. After forty-eight hours there are small funnel-shaped depressions in the gelatin, having yellowish points at their apex, while the gelatin begins to liquefy. Fragments of colonies having these characters are picked out with a platinum needle for microscopic examination, both in the hanging-drop culture and in cover-glass specimens. The Dunham solution flasks are incubated *for twelve hours only*, and are then probably cloudy from the rapid growth of the organisms, and the production of indole and

nitrites has proceeded sufficiently far to cause the appearance of the indole reaction (a distinct rose-madder tint) on the addition of a few drops of pure sulphuric acid.

Many other organisms besides Koch's comma also produce indole and nitrites in sufficient quantities to yield the indole reaction, but not in this time (twelve hours—it can often be obtained in five or six). The commas tend to form a delicate film on the surface of the medium. This should be examined, care being taken not to shake the flasks, so that the film may be preserved. In cases of true cholera the organism frequently cannot be demonstrated in the stool when the patient is on the way to recovery, so that the inability to demonstrate the organism in cases three or four days from the commencement of the attack must not be taken as evidence that the disease was not true cholera.

An agglutination reaction may be performed with the isolated vibrio. (Agglutination reactions with the patient's serum on a pure strain of the vibrio are said to be of doubtful value.)

Major Glen Liston (Rept. Bombay Bact. Lab., 1913) says the distinction between cholera and 'cholera-like' vibrios is based solely on their behaviour with a standard agglutinating cholera serum, as no morphological or cultural differentiation was discovered that could distinguish in a trustworthy way between the cholera and the 'cholera-like' strains.

Hæmolysis Test.—True cholera vibrios do not hæmolyse apparently even after prolonged contact, while several similar vibrios are hæmolytic. An emulsion of a young agar culture in 5 c.c. of normal salt solution is made, 0·1 c.c. of which emulsion is mixed with 0·9 c.c. of normal salt solution, and then a drop of a suspension of well-washed rabbit corpuscles added. Hæmolysis is generally apparent in two to twenty-four hours if the organism produces a hæmolysin, but some of the cholera-like vibrios described by Ruffer require forty-eight hours.

Saturation Test.—The saturation of a specific agglutinating serum with the homologous organism removes most or all of the specific agglutinin. (*a*) Ten loopfuls of a young agar culture of the isolated vibrio are mixed with

10 c.c. of a 5 per cent. solution of a highly agglutinating serum. After standing for two or three hours at room-temperature, the mixture is centrifuged and the clear supernatant fluid decanted. (*b*) The agglutinating power of the latter on the organism with which the serum was prepared is ascertained. If the organism treated in (*a*) is homologous with the organism with which the agglutinating serum was prepared, the decanted fluid will have lost most, or a considerable proportion, of its agglutinating power for the latter (Hewlett).

Fixation Test.—(See p. 20).

Pfeiffer's reaction (p. 22) is particularly valuable in the diagnosis of cholera.

Pathogenesis.—In Asiatic cholera urine is suppressed, and the copious and watery stools have the characteristic rice-water appearance due to flakes of detached epithelium. The most prominent symptoms are subnormal temperature, distension of the abdomen, and ultimately profound collapse.

The organisms are practically confined to the intestine, and are not to be found in other organs nor in the blood. Death may occur in twelve or even six hours after infection, or three hours after the first symptoms are noticed. The incubation period rarely exceeds two or three days.

Unless some heroic measure, such as a device to neutralise the acidity of the gastric juice, be employed, experiments on animals fail to produce cholera. Intra-peritoneal injections into guinea-pigs, though fatal, do not produce cholera. Young suckling rabbits are an exception, ingestion of the organism producing choleraic diarrhœa.

Occurrence and Distribution.—The disease is endemic in many parts of India, particularly the delta of the Ganges. In other countries its course may be traced along the ordinary lines of traffic, showing that it is carried by travellers. There has been no epidemic of cholera in the British Isles during recent years.

Cholera spreads most rapidly when the earth temperature is high; this happens chiefly when the ground-water is low, which is in accord with the observation of Pettenkofer that increase in cholera is often preceded by a fall in the ground-water.

Transmission of the disease may take place by means of water (as at Hamburg), by milk (rare), uncooked vegetables, or by fomites. The infection is confined to the bowel and stomach discharges, and is not found in the urine.

During outbreaks of cholera a number of persons showing only slight or no symptoms get infected at the same time as those who fall ill, and harbour the organism for some time. Haffkine has proved that such 'vibrio-carriers' can spread the disease.

As the vibrio offers little resistance to drying, it seems unlikely to be disseminated by dust. At the same time, it is readily capable of a saprophytic existence. Uncultivated vibrios die more speedily than cultivated ones and the duration of their life is shorter in the hot season than in the cold (Greig).

In some waters the cholera vibrio will live for considerable periods (see p. 225). Charcoal filters, once infected, have been known to continuously pollute water otherwise pure for many weeks, and cause grave epidemics.

The best-known instance of milk infection is that of the outbreak of cholera in the Gaya Gaol, in which it was surmised that flies carried the infection.

Under ordinary conditions, little or no toxin is found in cultures, but a powerful toxin (presumed to be an endotoxin) has been obtained by disintegration of the vibrionic structure.

Vaccine.—Haffkine's vaccine is prophylactic, not curative. An attenuated vaccine (prepared from cholera spirilla grown on agar at 38° or 39° C., over the surface of which a current of moist sterile air is passed) is injected. Five days later a stronger ('exalted') vaccine is administered, which does not exert its full power of immunisation till five days after inoculation. The virulence of the latter is obtained by passage through the peritoneal cavities of guinea-pigs. Both inoculations are made subcutaneously. Neither vaccine is sterilised or filtered, both living bacilli and their products being injected. The protection is of a very decided character.

Treatment by antisera has met with little success. The cholera immune serum is bacteriolytic, not antitoxic.

Cholera-like Vibrios.

Finkler-Prior Spirillum.—Ætiological significance uncertain. Is likely to be confounded with Koch's comma. It is occasionally found in the stools in English cholera (cholera nostras), cholera infantum, etc. The vibrio is rather thicker and longer than the Koch's comma, and has the following cultural characters: In gelatin stab culture liquefaction is rapid, extending in shape of a funnel to the bottom of the stab within forty-eight hours. On potato at 22° C. there is a slightly yellowish growth, and at 37° C. a rapid, slimy, yellow growth. Grown in peptone-water, a feeble indole reaction may be obtained after three days. A vibrio having very similar characters has been found in decaying teeth (Miller's Spirillum).

From cases of true cholera spirilla have been cultivated that closely resemble Koch's comma, yet differ slightly in cultural and other characters. Therefore 'Koch's comma' may be taken to be an organism with variable attributes, or more probably a group of organisms all capable of producing cholera, but not all exactly similar in character.

Sanarelli has isolated no less than thirty-two vibrios from water, morphologically distinct from each other, all of which gave a distinct indole reaction. Four of these organisms he found to be extremely pathogenic to animals, producing symptoms in guinea-pigs indistinguishable from those given by the true cholera spirillum.

Spirillum Metchnikovi.—This organism, which is pathogenic for fowls, pigeons, and guinea-pigs, but non-pathogenic for mice, closely resembles the cholera spirillum in morphological and cultural characters, even giving the indole reaction on the addition of sulphuric acid alone. The growth on gelatin affords a means of distinction from the cholera spirillum. On gelatin plates small white colonies form, which produce cup-like depressions on liquefaction in two or three days. In a gelatin stab liquefaction is more rapid than with the cholera spirillum, and takes place in the form of a funnel-like tube. It is more pathogenic for guinea-pigs than the cholera spirillum, and can be distinguished from the latter by the readiness with which pigeons succumb to septicæmia after inoculation, and by fatal results from feeding to fowls.

Spirillum Tyrogenum.

(*Syn.*, Deneke's cheese bacillus.) Forms no indole, is but feebly pathogenic for laboratory animals, and does not develop readily at blood-heat.

Spirillum Rubrum.

This organism, which is non-pathogenic, is found in water and garden earth. In broth long threads, with up to fifty twists, are formed. The shorter spirals are very motile. Colonies on gelatin out of contact with air and those on potato are red, and a red sediment is produced in broth.

Other spiral organisms, sometimes classed as Spirilla, are more correctly placed among the Protozoa, and will be dealt with later.

CHAPTER XIV

THE TRICHOMYCETES

The Trichomycetes are thread-forming organisms and form a group intermediate between the Schizomycetes and the Hyphomycetes. Much confusion exists as to the terminology of the class, the same term being used in different senses by authors. The following classification is convenient:

Leptothrix: No branching.

Cladothrix: 'False' branching.

Nocardia, or Streptothrix: True branching, with formation of rounded bodies, regarded as spores.

Actinomycosis.

Morphology.—*Actinomyces*, or ray-fungus, is a streptothrix occurring in three types in the colonies as they grow in the tissues—namely, filaments, cocci, and clubs. The filaments (seen better in cultures) are thin, measuring about 0·5 μ across, and are often of great length. The central protoplasm is enclosed in a sheath; the filaments, particularly in the centre of a colony, interlace, forming a network. In older filaments the protoplasm may be segmented, giving rise to a streptococcal appearance. These bodies are regarded as gonidia. The clubs are

involution forms, perhaps produced by resistance of the tissues. The filaments are sometimes ' acid-fast.'

When pus, sections, or teased-up specimens from human sources are stained by Gram's method, the filaments and gonidia are Gram-positive, while the clubs situated around the periphery and showing a radiating structure do not usually stain by Gram's method. Carbol-fuchsin and picric acid may be used, when the fungus stains red and the tissue yellow. In the bovine organism the clubs stain well by Gram and are well marked, clubs of the *hominis* variety being stunted. The prominent central filamentous network of the *hominis* variety is generally replaced by a mass of débris in the bovine type.

Cultural Characters.—In artificial media clubs are not found. The organism grows well, and for almost an unlimited time, on glycerin-agar and potato. Fully developed cultures on potato are peculiar and characteristic: a dull raised and wrinkled growth, of considerable thickness, of a bright sulphur-yellow or light chocolate colour, somewhat similar to the lichen commonly seen on apple-trees.

Occurrence and Distribution.—*Actinomyces* grows on grasses and cereals, particularly on barley, and especially on damp rich soils. Infection is due to the piercing of a mucous surface by a portion of a cereal bearing the fungus; possibly the fungus may also gain access to the system by inspiration.

It does not seem likely that cattle are infected by contact, and although some human cases have occurred after eating grains of barley, Wright is of opinion that actinomyces is normally present in the mouth, and that irritation caused by the foreign particle merely facilitates invasion of the tissues. The change of teeth in young animals is regarded as a period when risk of infection is greatest. Scirrhous cord, the fibrous tumour found on the end of the spermatic cord of the ox, is stated by Stockman to be caused by the entrance of the actinomyces by the wound of castration.

Pathogenesis.—Cattle are the principal subjects of the disease, but it also occurs in pigs, sheep, horses, squirrels, and man. In animals the disease is usually local and may arise in various parts of the body, the head and neck, particularly the tongue, being most

common sites. The disease commences with a swelling ('wen') that in due course ulcerates with the discharge of pus. In the tongue the growth of fibrous tissue makes it painful, hard, and immobile ('wooden tongue'), the tongue may protrude and ulcerate at its base. There is constant dribbling. The lower jaw-bone and the neck glands are commonly infected, the teeth often falling out from the former. The disease in man corresponds pretty closely to that observed in animals, but there is less tendency to localisation, to the abundant formation of connective tissue, and to the frequency of calcification. The tendency of the disease in man is to become chronic, and it is only by the implication of some vital organ or by the exhaustion following prolonged suppuration that the patient succumbs. The disease spreads by continuity, and no tissue seems able to resist its invasion. Besides this, secondary embolic foci may occur, perhaps the commonest seat being the liver.

Griffith (Report to L.G.B., New Series, No. 107, 1915), after examining a number of actinomycotic ox-tongues, supports the view that under the term actinomycosis a number of distinct conditions are included. Most of his specimens showed an organism probably identical with the actinobacillus of Lignières and Spitz.

If a little of the pus be allowed to run gently down the side of a test-tube, which is then held up to the light, the small yellow grains which will be visible may be picked out, placed on a slide, and pressed down with a cover-glass. It will transmit to the finger a sensation similar to that of squeezing a drop of solid fat, if the granule be taken from man; while if from an animal, the granule may be gritty, from calcareous infiltration. On examining the slide with a low power, a number of ovoid kidney-shaped masses are seen, which with a higher power show the characteristic club-shaped structure.

Mycetoma, or Madura Disease.

The foot is generally attacked, occasionally the hand, and rarely other parts. Three varieties are seen. The most common is the white, the black being less frequent, while the red variety is rare. The varieties are named after the colour of the granules occurring in the cavities.

The white form is produced by the *Streptothrix maduræ*,

distinguished from the actinomyces by its pathogenicity and cultural characters. It is not infective for rabbits, does not form yellow or black pigment in cultures, and does not liquefy gelatin. The cause of the black variety is uncertain, but it is credited to a hyphomycete.

Streptotrichosis.

In addition to actinomycosis and madura disease, other affections in which tubercles and suppuration are involved are caused by *Streptotricheæ*. The manifestations of some of these organisms strikingly resemble and are apt to be mistaken for tuberculosis. Some species, at any rate, are acid-fast and Gram-positive when the filaments are young, but older or degenerate threads are Gram-negative and not acid-fast.

Streptotrichosis may affect the mouth, neck, lungs, skin, kidneys, conjunctiva, appendix, and peritoneum. Foulerton (*Lancet*, 1910, i., 551, 626, and 769) says that cases show a preponderance of males, and the influence of occupation and other circumstances involving special exposure to the possibility of infection from a vegetable source is very noticeable. Eppinger's streptothrix (*St. Eppingeri*) belongs to Group II. of the *Streptotricheæ*, a class possessing acid-fast properties, and showing more active pathogenic action than the freely-growing species of Group I. and the slow-growing species of Group III. *St. Eppingeri* produces an infection which may be indistinguishable anatomically from a tuberculosis.

The occurrence of branching forms in *B. tuberculosis*, sometimes in typical actinomyces form, has led to a suggestion that it should be placed among the Trichomycetes. To quote Foulerton: 'There can be no reasonable doubt but that at one stage of its growth the bacillus of Koch is represented by branching mycelial threads of the streptothrix type. And this being so, the common "bacillary" forms represent the persistent rod segments of a streptothrix.' *Streptotricheæ* are commonly found on grasses, and among the reputed bacilli which display acid-fast properties are those having the characteristics of a streptothrix—*Bacillus phlei I.* and *II.* (Timothy-grass bacilli), and the cow-dung bacillus ('Mistbacillus') All three are pathogenic for guinea-pigs but grow readily on culture media.

Leptothrix.

Varieties of Leptothrix are common in the mouth and slime of the teeth. *L. buccalis* is a term perhaps comprising several species, some of which may cause dental caries. The threads have been thought to penetrate the tissue of the teeth, after the enamel has been acted upon by the acids generated by bacteria, including the fermentation of the food.

L. innominata (Miller), commonly found in the deposit on teeth, does not give the blue granulose reaction when treated with dilute sulphuric acid and iodine. This distinguishes the organism from *L. buccalis maxima* which gives the blue granulose reaction.

It is an open question whether all the Leptothrices in the mouth are not either bits of the same or stages in the life of the same.

Leptothrix epidermidis.—A non-pathogenic sporulating organism found in variolous crusts and on the skin between the toes.

Cladothrix Dichotoma.

This organism, which is common in natural waters, consists of long motionless filaments, sometimes a millimetre in length, which may possess pseudo-branches. The sheaths of the filaments are often coloured yellow, red, olive-green, or brown by oxide of iron. The *C. dichotoma* withdraws iron from water, and thus fixes it, often causing obstructions in iron pipes. On gelatin plates it forms small yellowish dots surrounded by a brown halo, and a depression due to the slow liquefaction of the gelatin. On agar it grows at 35° C. as a thick shining expansion, which adheres so closely to the medium that it is impossible to remove it without carrying away some of the agar. The growth has a tendency to form concentric rings, and sometimes becomes covered with a greyish efflorescence, which is dry and very brittle. The agar becomes brown in colour. All the cultures have a very strong mouldy smell.

Beggiatoa.

Beggiatoa are found in both fresh and salt water; in sulphur springs and brewery effluents they are

particularly in evidence. They are sometimes considered diagnostic of pollution with animal matter, but this is not invariably true. The growth may be white, grey, pink, red, or violet. The filaments contain sulphur particles, seen as highly refracting granules (see p. 6). They can decompose sodium sulphate in suitable organic solutions.

Crenothrix Kuhniana.

This is found in water containing organic matter or iron. It sometimes occurs in such great numbers that the water is unusable owing to the unpleasant odour and taste it produces. The organism produces a thick vegetable mass in the water, either brown or greenish in colour, frequently imparting a reddish or greenish tint to the water (see p. 6).

CHAPTER XV

THE BLASTOMYCETES

Blastomycetes, or yeasts, are round or oval unicellular organisms, consisting of granular 'cytoplasm' (hyaline in healthy, granular in old cells), surrounded by a wall of cellulose. One or more colourless lacunæ (vacuoles) are sometimes seen, particularly in healthy cells. A nucleus (or what is taken for one) may sometimes be seen. The salient character of this group is the method of reproduction by budding (gemmation): a bud is extruded from the parent cell, and, after enlarging, separates. *Torulæ* seem to be restricted to this method of multiplication, but *Saccharomycetes* can, in addition, reproduce by spore formation. Minute spherical bodies occur within the vacuoles in active movement, but their function is not known. In the absence of ample suitable nourishment, the contents of the cells become homogeneous, and then two, four, or, in the case of *Schizosaccharomyces octosporus*, eight, shining spots are seen, surrounded with a thick membrane. In the course of time these new cells (*ascospores*) become liberated by dissolution of the mother cell. On introducing these spores, which are 4 μ to 5 μ in diameter, into a saccharine

liquid, they form mature cells, which multiply, as usual, by gemmation. Dextrose, lævulose, mannose, and (sometimes) galactose, are the only hexoses fermentable by the yeast cell. Maltose, cane-sugar, malto-dextrin, and lactose are fermented, but different species vary in fermentation ability.

Saccharomyces Cerevisiæ.—Of the typical brewery yeast there are two main varieties, which are interconvertible: the typical English brewery yeast—a 'top' fermentation variety—and a yeast of the Continental lager beer brewer—a 'bottom' fermentation. The 'top' and 'bottom' varieties are known also as 'high' and 'low' yeasts respectively, as they ferment at high and low temperatures respectively. The rounded or slightly ellipsoidal cells are from 8 μ to 9 μ in diameter, and occur both singly and in short chains. Spores occur three or four together in a mother cell, each being 4 μ to 5 μ in diameter.

Fermentation with 'low' yeasts, in the manufacture of lager beer, takes place at 5° to 10° C., at which temperature other forms of yeast are inert. The process requires about fourteen days.

'High'-fermentation yeast consists of cells which are rather larger and more globular, and have a greater tendency to form branched chains than the 'low' yeasts. The temperature best suited for this fermentation is between 15° and 18° C. The reaction in the fermenting vats is much more violent than is the case with the 'low' yeasts. The rapid emission of carbonic acid brings the cells to the surface.

A soluble ferment (*invertase*) is present, which can be extracted by precipitating with chloroform, and which converts sucrose into invert sugar. Another enzyme occurs within the cells (*maltase*), which is capable of converting maltose into glucose.

Buchner, by grinding up yeast with kieselguhr and filtering under pressure, obtained a liquid which caused the evolution of carbonic acid and formation of alcohol in solutions of glucose. This he termed *zymase*, and regarded it as a solution of an intracellular ferment. Von Lebedeff prepares zymase by macerating 1 part of dried yeast with 2·5—3 parts of water, and filtering through paper after being allowed to remain overnight.

He claims for the solution greater activity and stability than that prepared by the usual method.

Zymase shows extreme instability, apparently due to a powerful proteolytic enzyme (*endotryptase* or *endotrypsin*), always present in yeast-juice, which, when digesting the coagulable protein, doubtless digests the zymase at the same time. The activity of zymase seems to be absolutely dependent on a co-enzyme, that withstands the temperature of 100° C. (thermostable) and is dialysable, but of which the constitution and function are unknown. Harden has shown the presence of phosphates is also necessary.

The juice extracted from yeast also contains rennin, a glycogen-hydrolysing enzyme, and a reducing enzyme which can liberate sulphuretted hydrogen from sodium thiosulphate and decolorise methylene blue. The yeast cell possesses special ferments capable of destroying zymase and its co-enzyme, also an anti-enzyme which prevents such destruction. See also Harden's 'Alcoholic Fermentation' (Messrs. Longmans' 'Monographs of Biochemistry').

S. cerevisiæ ferments saccharose, maltose, and dextrose. When the alcohol produced reaches 12 per cent., growth stops, and with 14 per cent. fermentation ceases altogether.

Saccharomyces Ellipsoideus.—The yeasts classed under this name are bottom fermentation forms, and are usually rounded or ellipsoidal in shape, but sometimes assume a sausage form. The cells average about 6 μ in length, are single or united in little branching chains. Two to four spores are found in a mother cell, each 3 μ to 3·5 μ in diameter (twenty-one to twenty-seven hours at 25° C.).

S. ellipsoideus I. is found on ripe grapes, and plays an important part in the fermentation of grape-juice.

S. ellipsoideus II. is a dangerous 'disease' yeast, producing yeast turbidity, in bottom fermentation breweries.

Saccharomyces Pastorianus.—This yeast—of which three varieties, known as I., II., and III., have been isolated by Hansen—is very polymorphic in shape. The cells are oval or sausage-shaped, and also occur in elongated, ellipsoidal, or pear-shaped forms. Two to four spores are usually found in a mother cell (twenty-five to twenty-eight hours at 25° C.). They take a part in many

spontaneous fermentations, and in the vinous fermentation usually succeed *S. apiculatus*. They are classed as 'wild' yeasts, the spores of which frequently occur in the atmosphere of breweries.

S. pastorianus I. is a 'bottom' form, and causes a disagreeable smell and a strong, bitter taste in beer.

S. pastorianus II. is a feeble 'top' form, and of no particular importance.

S. pastorianus III. is a top form, and is a dangerous disease yeast, causing yeast turbidity.

Saccharomyces Apiculatus.—This very common yeast can scarcely be termed a true saccharomyces, as no spore formation has yet been demonstrated. It occurs in wine fermentations and spontaneously fermented beer; on sweet succulent fruits, such as grapes, cherries, plums, gooseberries, etc. The cells, which are 6 μ to 8 μ long and 2 μ to 3 μ broad, have a most characteristic citron shape (hence the name), from the prominences at the end of which the budding takes place. It invariably appears at the onset of the vinous fermentation of grape-juice, but soon gives way to the *S. ellipsoideus* and *S. pastorianus*. It is a bottom yeast form, only gives rise to a very feeble alcoholic fermentation, and is incapable of fermenting cane-sugar and maltose.

Saccharomyces Mycoderma (Mycoderma Cerevisiæ).—The cells are oval, elliptical, or cylindrical, 6 μ to 7 μ long and 2 μ to 3 μ thick, united in freely-branching chains. It forms the skin, or 'mould,' on the surface of fermented liquids, without, however, exciting fermentation. When forced to grow submerged in a saccharine liquid, it gives rise to a small quantity of alcohol, but development is feeble. In liquids already containing alcohol it produces ethyl acetate.

Saccharomyces Exiguus.—Conical or top-shaped cells, 5 μ long, and reaching 2·5 μ in thickness, in slightly branching colonies. There are two or three spores in a row in each mother cell, but spore formation is scanty, and film formation does not occur. It is incapable of fermenting maltose. This yeast is often present in the after-fermentation of beer.

Saccharomyces Marxianus.—A yeast found on grapes. The cells are small, oval, or sausage-shaped, and form more or less kidney-shaped spores, the optimum development

of which is between 22° and 25° C. It only produces 1-1·3 volumes per cent. of alcohol.

Saccharomyces Anomalus.—Found in impure brewery yeast, on green malt, and on fruit. It is peculiar in that the spores are hemispherical, with a projecting rim, and are like a bowler hat. It forms but little alcohol.

Saccharomyces Pyriformis.—A yeast which, with a symbiotic bacterium—*B. vermiforme*—was found to cause the change in a solution of sugar and ginger produced by the addition of the 'ginger-beer' plant (Marshall Ward).

Schizosaccharomyces Octosporus.—Found on currants and raisins. In the genus *Schizosaccharomyces* of Lindner the cells are like yeast cells, but multiply by fission through a septum formed across the middle of the cell, and *not* by budding. Ascospores are also developed, in this particular species being usually eight in number.

Examination of Yeasts.

Hansen's scheme involves the isolation of a pure culture, and the observation of the time taken at various temperatures to form ascospores and 'films.'

1. **Microscopical Appearance.**—The growths, after growing in sterilised wort for twenty-four hours, are examined. When *S. cerevisiæ* and *S. pastorianus* are mixed or other varieties are present, little is to be learnt from a direct microscopical examination.

2. **Formation of Ascospores.**—The formation of spores in the saccharomycetes is regulated by the following conditions: (*a*) The cells must be placed on a moist surface, and have plenty of air. (*b*) Only young and vigorous cells can exercise this function. (*c*) The most favourable temperature for most species is about 25° C. (*d*) A few saccharomycetes form spores when present in fermenting nutrient liquids.

A small portion of a young and vigorous growth is transferred to a moist gypsum block, prepared as follows: To well-baked plaster of Paris distilled water is added until the plaster is nearly liquid; this is poured into a small mould of metal or paper. The blocks are allowed to set, dried, and sterilised. They are then laid in a shallow tray containing a little sterile water, the whole arrangement being kept well covered by a bell-jar.

Two sets of cultures on plaster blocks are made, one

set being incubated at 25° C., the other at 15° C. In a pure brewery yeast no ascospores will be detected under thirty hours and seventy-two hours respectively.

Hansen found that the formation of spores takes place slowly at low temperatures, more rapidly as the temperature is raised to a certain point; when this point is passed their development is again retarded, until finally a temperature is reached at which it ceases altogether. After a time, which varies with the species, roundish plasma particles appear in the cells. These are the first indications of spores. They become surrounded by a wall, seen more or less distinctly in different species. The spores may expand to such an extent that the pressure which they exert on each other whilst still enclosed in the mother cell develops the so-called partition walls. Later complete union of the walls may take place, so that a true partition wall results; the cell then becomes a compound cell, divided into several chambers. During germination the spores swell, the wall of the mother cell, originally thick and elastic, stretches thinner, finally ruptures, and then remains as a loose shrivelled skin, partially covering the spores, or else dissolves.

3. **The Formation of Films.**—With the pure cultivation, drop cultures are made into 4-ounce flasks, half filled with sterile wort, and protected from falling particles by a well-fitting cap. The films first appear as small opaque points, which gradually increase in size and then run together, forming irregular floating patches. As soon as the film becomes apparent to the naked eye it is examined. The film at length overspreads the whole surface of the liquid, and becomes adherent to the walls of the flask. Perfect rest of the liquid is a very necessary condition for the formation of films.

Hansen's Pure Culture Method.—*Böttcher's moist chamber* consists of a short glass cylinder or ring, cemented to the upper surface of a glass slip. A cover-glass rests on top of the ring. A small drop of water is placed at the bottom of the chamber. A mixture of yeast growth and sterile water is mixed with a suitable amount of wort gelatin, which has been previously liquefied at 30° to 35° C. A very small quantity of the mixture is spread in a thin layer on the sterile cover-glass and left under a sterile bell-jar to set. The edge of the ring of the

Böttcher chamber is painted with sterile melted vaseline, and on this the gelatin-coated cover-glass, infected surface downwards, is placed and pressed down, so that the chamber is completely sealed. The edge is painted with a melted mixture of 1 part of wax and 2 parts of vaseline, to prevent the cover-glass moving. The preparation is examined, the position of isolated cells is marked, and the chamber incubated at room-temperature till the colonies have become sufficiently large to be transferred to liquid media. With a Böttcher chamber with a ring of 30 millimetres diameter, twenty to thirty cells is a convenient number to have in the gelatin mixture. The method needs some practice to obtain results.

Torulæ (*vide supra*).—The *Torulæ* produce little or no alcohol in saccharine liquids. *Saccharomyces rosaceus*, *niger*, and *albus* are met with in air, and form coral pink, sooty black, and white growths respectively on potato and bread.

Yeasts in Sour Milk.—Herschell and Emerson agree with the use of a yeast in the preparation of Bulgarian soured milk for therapeutic purposes, not only to improve the flavour and to attack the milk-sugar after lactic acid has been formed, but also to inhibit the overgrowth of pathogenic and putrefactive bacteria. It is not unusual for the yeast to get the upper hand, and carry on the fermentation much too far.

Pathogenic Yeasts.—Blastomycetic dermatitis (blastomycosis) is sometimes seen in man. The disease may become generalised, and sometimes ends fatally. Yeasts have also been obtained from tumours, and some connection between yeasts and malignant tumours has been suggested. An outbreak of sore throat at Lincoln was thought by Klein to be caused by a yeast found in a throat. The yeast produced swelling of the throat, fauces, and larynx in rabbits.

CHAPTER XVI

THE HYPHOMYCETES

THE moulds or Hyphomycetes are multicellular organisms composed of filaments (hyphæ), which, when interlacing, form a mycelium. A mycelium may form a hard woody

mass (sclerotium). Lower members of the group have both asexual and sexual methods of reproduction, while among the high fungi sexual development is less evident.

According to the form of the seed-bearing organ, the moulds are divided into:

1. **Mucorinæ.**—The end of an aerial hypha swells into a knob, known as a columella, around which a spherical seed-capsule or sporangium forms. When ripe, the spores burst the enclosing membrane, and thus become free.

2. **Aspergillinæ** (Knob-Moulds).—The heads of aerial hyphæ are studded with spore-carriers, or sterigmata. Each sterigma bears a chain of spores (gonidia).

3. **Penicilliaceæ.**—Aerial hyphæ (goniodophores) branch at the apex forming *basidia*, on the terminals of which are the sterigmata, from which the conidia, or spores, are separated in the form of chains.

The most important members of these groups are the undermentioned:

Mucor mucedo.—A mould frequently seen on food-stuffs, particularly stale moist bread, and on animal excreta. It grows well on an acid medium, forming a white fur. It is not pathogenic.

Mucor rhizopodiformis forms a similar growth to the above. A culture on bread has an aromatic odour.

Mucor corymbifer forms a dense white fur on bread, resembling cotton-wool.

Mucor ramosus grows on bread and potato as a white fur, which soon changes to greyish-brown.

These last three mucors are pathogenic. Intravenous injection of their spores causes fatal disease in rabbits.

Aspergillus niger, A. albus, and *A. glaucus* grow upon bread, candied fruit, etc. The last two organisms grow best at blood-heat, when they soon overgrow the nutrient medium.

Aspergillus flavescens and A. fumigatus.—The former is distinguished by its well-marked fructifications and the greenish colour of its culture, the latter by its fine fructifications and ash-grey fur. On gelatin plates the filaments grow rapidly into the medium, causing liquefaction. Both organisms grow at blood-heat. Both are pathogenic, growing in various parts of the body—particularly the ear, producing otomycosis. They have been also found growing in the lungs and on the nasal mucous membrane.

The spores cause the death of rabbits on intravenous injection. Most cases of aspergillosis occur among bird fanciers, especially those handling pigeons. In the birds the disease takes a pulmonary form, and *A. fumigatus* is most frequently found.

Penicillium glaucum, the commonest mould, is seen as a pale bluish-green fur on jam and damp surfaces. It produces a peculiar musty odour. The mycelium consists of horizontally arranged straight or slightly undulating jointed filaments, from which the spore-bearing hyphæ stand vertically up, dividing at their upper ends into forks (*basidia*), from which fine processes branch off (*sterigmata*) in the shape of a hair pencil, and are segmented at their ends into rows of fine globular spores or conidia. The mould grows well on bread-pap in the form of a fur, white at first, but afterwards green. On gelatin plates fine threads diverging from a point, and not giving rise to sharply defined colonies, radiate over a considerable surface. The spore-bearing hyphæ which rise above the level of the gelatin are put in motion by air currents, when the spores disperse. The earliest formation of spores takes place in the centre of the colonies, and is indicated by a green colour. The gelatin is liquefied.

The volatile arsenical compound, which is given off by arsenical wall-papers to cause poisoning in the tenants of the room, is almost certainly liberated from its combination in the pigment by micro-organisms thriving in the paste. *Penicillium*, *Mucor*, and *Aspergillus* are found to be capable of so acting, most marked powers being given by *P. brevicaule*. Gosio has elaborated a test whereby *P. brevicaule* is used for the detection of minute amounts of arsenic (see Glaister's 'Arsenic Gas Poisoning').

Brown mould is brownish-yellow in colour, and is distinguished from *P. glaucum*, which it otherwise resembles, by its closely-felted mycelium, the hyphæ being scanty, ramified, and segmented. It grows on gelatin, which it quickly liquefies. According to Trelease, this mould is identical with the *Cladothrix dichotoma* (*q.v.*).

Microscopical Examination of Moulds.—Moulds cannot be easily moistened with water, owing to the presence on their surface of a very thin layer of fat. Alcohol to which a little ammonia has been added removes the fat, after which they can be mounted in glycerin or glycerin and

water. Löffler's methylene blue stains the filaments of the mycelium and hyphæ, the spores remaining unstained. For permanent preparations moulds are mounted in glycerin jelly, the cover-glass being ringed with varnish.

Culture of Moulds.—The moulds grow on nutrient gelatin and agar, on potato and bread-paste. The best media are glucose agar, wort gelatin, and wort agar.

Fermentation by Moulds.—Some species of *Mucor*, when immersed in a fermentable saccharine liquid, such as wort, very quickly change their appearance: the submerged hyphæ swell irregularly, and a large number of transverse septa appear, which divide them into barrel-shaped or irregular cells, filled with highly refractive plasma; these cells seem to multiply by budding, like true yeast. If, then, the above-mentioned cells are brought to the surface of the liquid, or otherwise under aërobic conditions, they are again able to develop the typical mould form. The most active fermentative power is possessed by *Mucor erectus*, which in ordinary beer-wort can be made to yield up to 8 per cent. by volume of alcohol. Another form, the *Mucor* (*amylomyces*) *Rouxii*, obtained from a Chinese rice fermentation, is used commercially for the conversion of starch into alcohol.

Oïdiaceæ.

The *Oïdiaceæ* are generally classed among the Hyphomycetes. No special spore-bearing organs are present, but the hyphæ bud at the extremities, forming spores.

Oïdium lactis, found in sour milk and butter as a white fur, grows on gelatin without liquefaction, producing a smell of sour milk. On agar it grows in the form of little stars, which then overgrow the medium. In a stab culture the fibres of the mycelia permeate the medium. *O. lactis* grows very readily in milk, which it does not change in any special way. It is not pathogenic to man or animals.

Oïdium albicans grows on the mucous membrane of the mouth and pharynx of children, producing thrush. If one of the white patches be removed and teased up, the filaments and yeast-like cells can be distinguished. It does not flourish in an alkaline medium, but can be grown on milk, bread, gelatin with liquefaction, agar, and potato. On all these media it produces a very copious growth,

showing yeast-like cells, the growth on potato being a whitish, thick, raised patch. After an intravenous injection rabbits die in about thirty-six hours, with their viscera full of the mycelium.

Gibbons says sometimes it causes pruritus and Bahr (*Medical Press*, October 7, 1914) describes it or a similar organism as occurring in the tongue lesions of sprue.

Oidium carnis (see p. 209).

Sporotrichon Beurmanni.

This organism may attack practically any of the tissues of man. The disease (Sporotrichosis) simulates tuberculosis and syphilis and produces pus. It can seldom be found in smears but is readily cultivated. Gougerot (*Medical Press*, October 7, 1914) recommends the pus from closed lesions being inoculated on to glucose gelatin and kept at room-temperature. He says that colonies of sporotrichum make their appearance between the fourth and the twelfth day; white at first, they soon become brown and then chocolate colour. He likens the colour and the wrinkling to mountains on a relief map or the cerebral convolutions. A brownish halo surrounds the colonies. Gougerot says infection takes place through scratches with thorns or with knives used for peeling potatoes, for example, and the ingestion of uncooked vegetables. Animals may convey infection.

The organism produces filaments and spores, and is thought to be a hyphomycete.

Ringworm.

American authorities include the forms of ringworm in a class called the *Keratomycetes*, owing to their power of living on the keratinised products of skin, which they break up and digest by means of keratolytic ferments. It rarely affects sheep and pigs, and in cattle is usually confined to the head and neck. In France it is frequent among horses. Dogs and cats are affected, and frequently transmit the disease. In the human subject most forms of ringworm disappear before the host reaches twenty years of age.

Two varieties at least of the disease are to be distinguished:

Microsporon Audouini is met with in young children. Sequeira (*School Hygiene*, August, 1912) found it present in 89·8 per cent. of ringworms in children. Hewlett says that it never attacks the scalp of adults, never affects the beard or nails, is very intractable, and frequently epidemic. It occurs as a whitish sheath round the stumps of broken hairs. Microscopically the parasite appears as round or ovoid spores measuring 3 μ to 4 μ in diameter.

The second form comprises two varieties. In one, which is exclusively of human origin, the spores are met with chiefly in the interior of the hairs, and hence is termed the *endothrix* variety. It occurs in late childhood (10·2 per cent. of children's ringworms [Sequeira]). In the *ectothrix* variety, which principally affects the beard and nails, and is derived from animals, the spores lie on the exterior of the hairs. In both the spores are large, measuring 4 μ to 12 μ in diameter, and the parasite is known as the *Trichophyton megalosporon endothrix* and *ectothrix* respectively.

The fungi can be readily cultivated on all the ordinary media, but beer-wort gelatin and agar are to be preferred, and the best of all is a maltose agar (Blaxall's English Proof Agar), composed of peptone 1 gramme, maltose 4 grammes, agar 1·3 grammes, and water 100 c.c. The growths are white and fluffy, and rapidly liquefy gelatin, the different varieties showing certain differences, and microscopically they consist of a tangled mycelium, with spores.

A pure culture may be often obtained by chopping up an affected hair and strewing it over an agar plate. After incubating for three to seven days at 24° or 30° C., colonies appear as little, fluffy, whitish spots. To diagnose a case of ringworm, it is generally sufficient to examine one of the suspected hairs under a low power ($\frac{1}{4}$ inch), when it will be found to be covered with spores. To facilitate examination, the hair may first be soaked in alcohol and ether and then in 10 per cent. caustic potash solution. If the patch itself is examined, spores will be found on the surface, while a little below will be seen a matted mass of mycelial branching. The organism may be stained with fuchsin or by Gram's method, and permanent preparations (unstained) may be mounted in glycerin jelly.

Gilbert Brooke states that *M. Audouini* and *T. endothrix* are rare in the tropics, and thinks that dhobie (washerman's) itch is caused by *Tinea ectothrix*. The success of X-ray treatment lies not in any power to destroy the ringworm spores, but in a copious defluvium of hairs from the part exposed, leaving a follicle free from disease. Germicides are recommended as an accompanying treatment.

Strickler and Kolmer (*Journal Amer. Med. Assoc.*, July 17, 1915) found that on injection into the superficial layer of the skin of the arm of children suffering from this disease of 0·05 c.c. of a suspension of dead ringworm fungus in salt solution, usually there occurs a local reaction. These authors recommend a vaccine.

Microsporon Furfur.

This organism, which is found in pityriasis versicolor, probably belongs to the same family as the *T. tonsurans*. It occurs as masses of short threads and clusters of oval refractile spores in the scales of the skin, but has not yet been artificially cultivated.

Achorion Schönleinii.

The yellow cup-shaped crusted masses of fungus in favus are caused by *A. Schönleinii*. The disease affects man, dogs, cats, caged rabbits, mice, and rats. In the last two cases it is commonly fatal. In the fowl lesions occur mostly on the comb and wattles as a grey crust ('white comb'). Favus can be transmitted from animals to man. *A. Schönleinii* in the earlier stages is indistinguishable from the *T. tonsurans*, but soon assumes the honeycomb appearance. It grows on all ordinary media except milk. Gelatin is liquefied. On agar the colonies appear distinctly in forty-eight hours. They are surrounded by a fine fringe of threads. On blood-serum star-shaped colonies are formed, which radiate out from the centre, producing a flower-like appearance. The medium is not liquefied. It also grows well on bread and potato.

CHAPTER XVII

THE PATHOGENIC PROTOZOA

THE Protozoa are unicellular organisms belonging to the animal kingdom. Reproduction generally takes the forms of simple fission and spore formation, but complex life-cycles, involving the alternation of sexual and asexual phases, occur in some. A conjugation of two dissimilar cells (gametes) may take place to form a *zygote*. Gametes fulfilling a duty analogous to that of spermatozoa are called *microgametes*, the other variety being *macrogametes*.

The following is one classification of the Protozoa:

Sarkodina.—The Amœbæ, the only type of this division parasitic for man, have no cell-wall, movement is slow, and takes place by the projection of temporary limb-like processes (pseudopodia). Multiplication is by fission or spores.

Mastigophora.—Flagellated forms, including trypanosomes and probably spirochætes.

Sporozoa.—Passive and exclusively parasitic organisms, reproducing by spore formation, including malarial parasites, *Coccidium* and *Piroplasma*.

Infusoria.—Actively motile ciliated protozoons, with definite cell-walls, and small micronucleus participating in conjugation, and a vegetative macronucleus. Includes *Balantidium*.

Parasitic Amœbæ.

Three Amœbæ, known as **Entamœbæ**, are parasitic for man:

Entamœba buccalis occurs in dental caries.

Entamœba coli is an apparently harmless, and not infrequent, inhabitant of the bowel.

Entamœba histolytica is found in the fæces in the form of chronic dysentery common in the tropics, and in the pus of tropical abscess of the liver, a not infrequent sequela of this form of dysentery. *A. histolytica* measures about 15μ to 50μ in diameter. The pseudopodia are short and blunt, and vacuoles, a nucleus, and highly refractile granules are to be seen in the endosarc, often together with blood-corpuscles, pus cells, and bacilli. Amœbæ can be separated from ingested bacteria by a 20 per cent.

solution of sodium carbonate; but in order to obtain growth, it is necessary to use an agar medium, and supply the organisms with living bacteria. In the fæces spore formation takes place. The disease is supposed to be spread by drinking water and raw foods. Jordan says that sand filtration will not remove Amœbæ from water.

The Trypanosomes.

The trypanosomes have a somewhat elongated eel-like form, the average length being from 20 μ to 35 μ. They belong to the Flagellata class of the Mastigophora division of the Protozoa. The description of *T. Brucei* given below is with slight modifications accurate for other species. Trypanosomes have no mouth, nutrition being effected by the imbibition of soluble nutrient material. When living they move rapidly about in the fluids of the body, being propelled by a flagellum, by the undulations of a fin-like structure—the undulating membrane—and by alternate contractions and relaxations of the body protoplasm. They reproduce by a simple longitudinal division. While some authorities aver that no true conjugation has been discovered, others differentiate a bulky, granular, slow-moving organism as the female, and a slender, active one as the male. These sexual types are said to conjugate in the *Glossina* or other invertebrate host and a transitional form between the male and female varieties is regarded as an indifferent form, or as a daughter cell split off from a fertilised female.

Trypanosoma Gambiense is found in the glands, cerebro-spinal fluid, and blood in sleeping sickness. The organism is conveyed by a tsetse-fly * (*Glossina palpalis*), which feeds on an infected being, and conveys the trypanosome in its alimentary canal till it bites a healthy subject (frequently on the back of the neck), and thus causes infection. An exogenous cycle appears to take place in the gut of the fly, involving differentiation into sexual types and fertilisation of the female. The fly remains infective after feeding on infected blood for ninety-six days, and possibly for entire life. *Glossina palpalis* does

* Tsetse-flies belong to the same order as the house-fly (*Muscidæ*), which they resemble in general aspect, but when at rest the wings fold completely over each other, like the blades of a pair of scissors. The flies are viviparous, extruding single annulated adult larvæ.

not feed on ordure, but frequents the brushwood near water where birds and fishes congregate, and from these it derives the blood it must have every two or three days. It feeds on fishes lying on the surface, hippopotami, and crocodiles, its proboscis being able to pierce even through elephant-hide. *Glossina fusca* and mosquitoes of the genera *Mansonia* and *Stegomyia* are possibly important auxiliaries in conveying the disease.

The organism may be found by direct examination of a wet blood-film or in the juice of the enlarged glands. The parasites are often difficult to find in the cerebro-spinal fluid and blood. They may be absent from the blood and present in the cerebro-spinal fluid, and *vice versa*.

The inoculation of monkeys with the blood or cerebro-spinal fluid may detect the disease when direct examination is negative. A count of the white cells of the cerebro-spinal fluid shows an increase in the small lymphocytes (Broden and Rodhain).

Two distinct and separate forms of sleeping sickness are recognised: the Uganda form, which is violently epidemic, and the variety prevalent in Nyasaland and Rhodesia. The latter is almost certainly not epidemic, and is caused by a different trypanosome, which is carried by *G. morsitans*, a tsetse-fly that appears to be independent of water.

Yorke and Kinghorn found that a large proportion of the wild game in Equatorial Africa, though apparently quite healthy, are veritable reservoirs of trypanosomes constantly infecting the tsetse-flies who feed upon them; and the tsetses in turn infect man. In the case of the Rhodesian or Nyasaland form, the Inter-Departmental Committee on Sleeping-Sickness concluded that 'the evidence is conflicting as to whether the wild animals which are a reservoir of the disease affecting domestic stock are a danger to man.' And further, that in the case of the Uganda form 'the part played by wild animals is of minor importance, as compared with that played by man himself,' as a reservoir from which the fly derives the infection.

Sleeping-sickness may have a conjugal origin. Monkeys, dogs, and rats are susceptible, but donkeys, oxen, goats, and sheep, up to the present, have shown themselves

refractory. There is no evidence that a previous attack of sleeping-sickness gives any immunity. Trypanosomes surviving medication with arsenic compounds acquire a tolerance of them.

Trypanosoma Brucei.—Domesticated horses, cattle, and dogs entering a 'fly country' are liable to nagana, or 'fly disease,' the ætiological agent of which is *T. Brucei*. This parasite has a somewhat slender, spindle-shaped body, about 15 μ to 20 μ in length, bluntly pointed posteriorly, and prolonged into a long, delicate flagellum at the anterior end. On one aspect of the organism is a delicate, undulating membrane, commencing near the posterior extremity at a micro-nucleus, or blepharoblast, and becoming prolonged into the flagellum. Near the centre of the organism is a macro-nucleus, and the protoplasm is refractile and finely granular, and contains a vacuole. The appearance of this hæmatozoon in the blood is signalised by a rise in temperature; the incubation period is from seven to twenty days, after which period the hæmatozoa may be found swimming actively among, and apparently 'worrying,' the corpuscles, red blood-corpuscles becoming very largely reduced in numbers. *G. morsitans* transmits the parasite. Wild game harbour the parasite without showing any sign of the disease—in a happy expression of Bruce, they act as 'reservoirs,' and removal of the big wild herbivora from a district considerably lessens the incidence of the disease.

Trypanosoma Evansi is found in *surra*, a disease of horses and camels in India. The parasite is more active than *T. Brucei*, but is regarded by some as identical with it.

Trypanosoma equinum occurs in an equine disease prevalent in South America, *mal de Caderas*, the prominent symptom of which is paralysis of the hindquarters.

Trypanosoma dimorphon, or dimorphum, occurs in a trypanosomiasis of horses in Senegambia. Two forms, long ands hort, occur, and by the prolongation of the protoplasm to the tip of the flagellum a free process is eliminated.

Trypanosoma Theileri measures up to 65 μ in length, and is found in South African cattle affected with galziekte.

Trypanosoma Transvaaliense measures up to 50 μ long and up to 6 μ thick. It is found in South African cattle.

Trypanosoma equiperdum is found in dourine (*mal du coit*) in horses, and is transmitted by coitus.

Somewhat similar organisms are frequently found in the sewer-rat (*T. Lewisi*), apparently doing little or no harm, and in many other mammals, birds, reptiles, and fishes. *T. Lewisi* is conveyed by a rat-flea. Nöller and also Wenyon say that it is not the act of biting which infects the rat, but that infection is brought about by the rat licking up the fæces passed by the flea while feeding.

The trypanosomes stain well by Leishman's method. By cultivating on a blood agar (ordinary agar, with an equal quantity of sterile defibrinated rabbit's blood) many of the trypanosomes may be grown—*T. Lewisi* with ease, *T. Brucei* with more difficulty.

Leishmania Donovani.—The spleen and liver of persons suffering from kala-azar (tropical febrile splenomegaly, dumdum fever) contain Leishman-Donovan bodies. They are round or oval bodies, measuring 2·5 μ to 3·5 μ. Some sort of cuticle is apparently present, and there are two chromatin masses—a large one, staining pale red with Leishman's stain, and forming part of the periphery of the parasite; and the other a small one, staining deep red with Leishman's stain, situate opposite the other, in the short axis of the parasite. Donovan suspected a reduviid bug (*Conorhinus rubrofasciatus*, de Geer) as being a possible transmitter of the disease. Patton has described the complete development of the parasite in Indian and European bed-bugs. A parasite indistinguishable from *Leishmania Donovani* was found by Nicolle in the splenic smears of Tunisian dogs, but Donovan failed to find it in an enormous number of smears from Madras dogs. Nicolle's parasite is considered to be a separate species and is called *Leishmania infantum*.

A goitre affection occurring in Brazil is said to be a trypanosome infection. Donovan tritely remarks that there appears 'to be no limit to the existence of the parasitic flagellates in animal organisms, but what is more astonishing and subversive of previously held views is the occurrence of these parasites in the latex or milky juice of plants.' Lafont found Herpetomonas (Leptomonas) in the latex of *Euphorbia pilulifera* in Mauritius, and Donovan discovered these flagellates—small, narrow forms—in the latex of the same plants growing in Madras.

The organisms differ from the known flagellates parasitic on animals, and will doubtless be placed in a new genus, for which the name of Phytomonas has been suggested by Donovan.

The Spirochætes.

Authorities are divided as to whether the members of this group should be regarded as Bacteria or Protozoa. The presence of a definite nucleus, blepharoblast, undulating membrane, and multiplication by longitudinal division suggest a protozoan nature, but some observers have thought the flagellum to be of a bacterial type in *S. recurrentis*, and Jordan states that this organism multiplies by transverse fission.

Spirochæta recurrentis (Spirillum Obermeieri) is an actively motile, delicate, spirillar filament, found in the blood-plasma in relapsing fever during the febrile period. It measures about 8 μ long and about 0·3 μ thick, and is pointed at the ends. Monkeys can be inoculated, but, with the exception of rats, other animals are immune. Bed-bugs and pediculi are supposed to transmit the disease. Darling found granular forms in the tissues of animals infected with the spirochæte of the relapsing fever which occurs in the Isthmus of Panama, which granules he suspects to be biogenetically connected with the spirochætes, and inoculation of such tissue, apparently completely free from spirochætes, was followed by infection.

Spirochæta Duttoni occurs in the body of the tick (*Ornithodoros moubata*). Clumps of chromatin granules, considered by Leishman to be biogenetically connected with the spirochæte, are found in the Malpighian tubes of the tick (*Lancet*, 1910, i. 12). These ticks, by feeding on a man or monkey, produce tick-fever.

A fowl spirochætosis occurs in the Soudan, and is transmitted by *Argas persicus*. **Spirochæta anserina** occurs in geese. Fantham, Chalmers, and O'Farrell found a spirochæte in a form of bronchitis endemic in some parts of the Soudan.

Spirochæta pallida, or **Treponema pallidum.**—This actively motile spiral-shaped protozoon is very thin and varies from 4 μ to 14 μ in length, with an average thickness of 0·25 μ. The ends taper. Six to eight spirals are

generally seen, though more (up to twenty) are often found. The existence of a flagellum is uncertain. The virus is destroyed by exposure to X rays or to a temperature of 48° C. for half an hour.

T. pallidum is now accepted as the cause of syphilis. It is found in the primary sore and neighbouring lymphatic glands, in the papular and roseolar eruptions and condylomata of the secondary lesions. It is also found but with some difficulty and only in small numbers in gummatous lesions. Considerable numbers of *T. pallidum* are found in the liver, lungs, pancreas, spleen, thymus, and adrenals of syphilitic fœtuses. Noguchi demonstrated the presence of this organism in the brains of patients dying of general paralysis; Stoddart holds the view that the treponeme of general paralysis is not absolutely identical with that of syphilis. Moss has suggested that there is a special general-paralysis-producing variety of the syphilitic organism.

McDonagh thinks the protozoon enters the body while in the spore phase, and that *T. pallidum* probably represents the end phase of the life cycle and is a male. McDonagh says that where extracellular development (only described in chancres and condylomata) occurs, the immature treponeme that is first formed resembles the *refringens* type.

T. pallidum can be cultivated by Noguchi's method; material for inoculation is obtained not from human lesions, but from the artificially infected testicular tissue of the rabbit. This is introduced into serum water to which a piece of sterile rabbit tissue (preferably kidney or testicle) has been added. The surface of the medium is covered with a layer of sterile paraffin. Once primary culture has been effected strict anaërobiosis is not essential; and the organism can in subcultures be transferred to solid media such as ascitic fluid agar.

The treponemes can be separated from the bacteria that usually contaminate the primary culture by letting them grow through filters which retard the passage of other organisms, or by growth in stab cultures where the treponemes grow away from the line of puncture into the surrounding medium while other bacteria fail to do so.

In ulcerating syphilitic lesions **Spirochæta refringens** sometimes accompanies *T. pallidum*. McDonagh (*Lancet*,

1910, i. 920) says the question of distinguishing the *T. pallidum* from the *S. refringens* need not arise, since the latter is rare in the secretion of a chancre, and does not occur in other venereal diseases until a stage so late that a wrong clinical diagnosis is impossible. *S. refringens* is thicker, has fewer and wider turns to its corkscrew, and is more refractile; while on the other hand, the *T. pallidum* is finer, has more turns, tapers at both ends, and appears dead white.

The secretion of a primary sore examined by means of the 'dark ground illumination' is said to hardly ever fail to reveal the *T. pallidum*. A chancre is cleansed by washing with normal saline and a drop of clear exudate squeezed out. This is taken up in a capillary tube, placed on a microscope slide and covered with a cover-glass. A drop of cedarwood oil is placed on the under surface of the slide, and contact made with this and the upper surface of the parabolic dark ground condenser. The preparation is examined with a $\frac{1}{6}$-inch objective.

The 'dark ground illumination' allows no rays of light to reach the eye of the observer, except those reflected by certain objects, such as the treponeme, under observation. The treponeme is seen twisting its corkscrew-like form, which is brightly illuminated, across the dark, non-transparent background. Neither form nor size is distorted. *T. pallidum* appears moderately stiff and rigid. Movements of flexion and oscillation are not so marked as in some allied species. With the exception of *S. dentium*, *T. pallidum*, almost alone among the spirochætes, retains its spiral arrangement not only during movement but also when at rest. In other words, its spirals represent a permanent arrangement. Dark background illumination can be carried out almost as well on dried as on living preparations.

Material for microscopic preparations may be obtained by the method recommended by Phillips and Glynn. The lesion is cleansed with cotton-wool, rubbing it with wool, and then swabbing with wool soaked in methylated spirit. At the end of four minutes the spirit is wiped off, and the clear serum which exudes collected in a capillary tube.

The organism may be stained by Giemsa's stain: Azur II. eosin, 3 grammes; Azur III., 0·8 gramme;

glycerin, 250 c.c.; methyl alcohol, 250 c.c. Blood films or smears from chancres are fixed in alcohol for fifteen minutes and then stained in Giemsa stain, diluted to the extent of 1 drop in 1 c.c. of distilled water, and made alkaline with dilute potassium carbonate solution, for five hours or longer. The slides are washed in distilled water and dried. *T. pallidum* is usually stained a light rose-pink and other spirochætes and organisms blue.

Burri's Indian-ink method (p. 48). Günther-Wagner ink is sterilised by steaming. Sommerville recommends centrifuging the ink until a film made from the upper portion of the column appears under the $\frac{1}{12}$-inch objective quite homogeneous. Equal quantities of exudate and ink are mixed and spread on a slide. The preparation is allowed to dry and examined by the oil-immersion lens without a coverslip. The *T. pallidum* appears as a white, wavy thread in a dark field. Sometimes treponema cannot be discovered in chancres after repeated examinations and the cases ultimately prove to be of syphilis.

The Wassermann reaction (p. 21) is used for diagnosis, and appears to be of most value in the secondary stage of the disease. It is inadvisable to place reliance on a negative reaction in the primary stage, but as the method improves the cases of definite untreated syphilis in which a negative reaction occurs are becoming fewer and fewer. The reaction is positive in both the blood and cerebro-spinal fluid of general paralytics but not at every examination. D'Arcy Power says it is not usually positive until five to eight weeks after infection when the disease has ceased to be local and has become generalised in the body. McDonagh thinks it doubtful whether our present interpretations of its results are correct, and succeeded in making a reaction positive or negative at will. H. C. French states that the test may be negative, and later positive, or *vice versa*, without any material change in the immediate surroundings of the patient.

Positive Wassermann reactions have been found in cases of non-syphilitic (soft) chancre (French), and also in alopecia areata, gonorrhœa, yaws, leprosy, malaria, puerperal eclampsia, lupus erythematosus, trypanosomiasis, enteric fever, tumours, herpes, aortic disease, relapsing fever, in some persons under narcosis and in serum from dead bodies. Positive results of a transient

nature have also been reported in pneumonia and scarlet fever.

Luetin. Cultures of *T. pallidum* are ground up with normal saline, heated to 60° C. for an hour and 0·5 per cent. phenol added. Luetin is injected into the skin of the arm and a positive reaction consists of the production at the site of inoculation in forty-eight hours of a diffuse erythema with sometimes the development of a papule or pustule later on. In non-luetic cases a slight erythema occurs. A positive result means no more than that the patient has had syphilis, it does not indicate that the disease is actually active.

As a result of the administration of arsenical preparations in treatment, arsenic-resistant treponemes may be produced.

The Malarial Parasite.

Plasmodium malariæ or *Hæmamœba malariæ* is conveyed from man to man by mosquitoes of the genus *Anophelina*, the commonest species of malaria carriers being the *Anopheles maculipennis*, *A. funestus*, *A. costalis*, *A. sinensis*, and *A. argyrotarsus*. When at rest the proboscis, thorax, and abdomen of the Anopheles form a straight line, with the proboscis almost touching the wall, and thus form an angle with the wall or other resting surface. The resting *Culex*, on the other hand, rests with its body parallel to the resting surface. The ova of the Anopheles are much smaller than those of other mosquitoes, and are boat-shaped. Down the centre of the body of a larva run two breathing tubes, the opening of which is in the anterior part of the last segment, which necessitates them floating horizontally at the surface of water. The larvæ of other mosquitoes hang down from the surface. The female alone bites, generally at night, and, in the event of the subject being malarial and the parasite being ingested, its development is complete in eight or ten days, when it is ready for successful inoculation into a healthy subject. After the mosquito bite the organism enters a red blood-corpuscle, being at first a small, pale, nebulous, ill-defined body, which grows in size and alters in shape, sometimes to a ring-like form, developing black pigment granules, which exhibit slow movements, and tend to concentrate while the amœboid

movements slacken. Ten to twenty segments develop, which form round a central clump of pigment, like a rosette, the blood-corpuscle breaks down, and the segments, which have now become spherical, are liberated. Some enter red blood-discs and repeat the cycle (the human phase, endogenous or asexual cycle), the bodies in the corpuscles being known as amœbulæ or myzopods, and the liberated spheres as sporocytes or merozoites. The liberation of the latter is coincident with the appearance of a paroxysm.

Varieties of the Malarial Parasite.—Varieties of the parasite supposed to be biologically distinct organisms are associated with respective types of fever, which are distinguishable clinically.

(*a*) *Quartan.*—This is a benign variety, and depends on a parasite (*Hæmamœba malariæ*) which takes seventy-two hours to pass through its cycle of development. The parasite is feebly amœboid at first. The pigment is abundant and coarse-grained, and the symmetrical rosette that forms next produces six to twelve merozoites. The red corpuscles invaded by the parasite do not become decolorised or markedly enlarged.

(*b*) *Tertian.*—The cycle of development of this parasite (*H. vivax*) takes forty-eight hours for completion. The organisms within the corpuscles show much greater movement than in the quartan type. The pigment granules are fine, and show much movement. The sporulating body consists of fifteen to twenty segments, which form a mulberry mass. The corpuscles are frequently decolorised and hypertrophied.

(*c*) *Malignant Tertian.*—This form (*Hæmomenas præcox*) requires about forty-eight hours for development. The organism is relatively minute, and is very actively amœboid when young, the parasite assuming a 'signet-ring' form after a time. Its more advanced or sporulating stage is completed in the bloodvessels of the deeper viscera.

(*d*) *Malignant Quotidian.*—The cycle of development of this variety takes twenty-four hours. The parasite is always small, even in the adult state, and frequently assumes the 'ring' form in the corpuscle. The spores are generally formed by irregular segmentation.

There is some disagreement as to the existence of

different parasites in the malignant form, and they are often classed together as 'the parasites of æstivo-autumnal fever.'

The main distinction between the benign and malign species is that in the case of malign parasites flagella are produced from crescent bodies, while in the case of the benign parasites they are produced from simple spheres. Crescent bodies are always found in malign fevers, though not in the early stages. They continue in the blood for many days or weeks, and are much less affected by quinine than the other varieties.

The Sexual Phase.—Within the human host asexual development alone takes place, but a sexual phase occurs in the mosquito. Amœbulæ become specialised into male cells (microgametocytes) and female cells (macrogametocytes). The microgametocytes develop four to eight filaments (gametes), which break away to fertilise the large spherical and granular macrogametes, forming a 'zygote,' spoken of at this stage as a 'travelling vermicule.' This becomes encysted in the stomach wall. This grows, divides into eight to twelve 'zygometres,' each of which becomes a spherical 'blastophore' and develops numerous slightly sickle-shaped, radially disposed bodies, or zygotoblasts. When mature the blastophore disappears, the capsule ruptures, the zygotoblasts are poured into the body cavity of the insect, and make their way to all parts, including the salivary glands, whence they are discharged by the middle stylet of the proboscis when the insect 'bites' its next victim. The 'blasts' then become attached to corpuscles, and develop into the ordinary form of malaria parasite.

Flagellated bodies do not, apparently, occur in fresh human blood, but are found when wet specimens have been under the microscope for some time. The pigment granules become arranged in the form of a central ring, and afterwards show a peculiar vibratory movement, which is apparently caused by the flagella which have formed within the sphere. At this stage the flagella shoot through the walls of the envelope of the sphere. The flagella, which are usually from three to six in number, are very delicate, actively moving filaments often having a bulbous swelling at their ends. They frequently break away and swim free in the blood.

Examination of Fresh Blood.—A droplet of blood (p. 46) is touched with the centre of a cover-glass, which is immediately placed on a slip. If cover-glass and slide are clean the blood runs out in a delicate film. The slide is examined with $\frac{1}{12}$-inch immersion lens, in a not too bright light, and the interior of every corpuscle is searched for specks of black pigment, surrounded by a pale, hyaline, slightly or markedly amœboid, substance; also for smaller, pale, unpigmented, hyaline, and more actively amœboid bodies in the same situation.

Stained Blood Preparations.—A film is made (p. 46), allowed to dry spontaneously, fixed by immersion in a mixture of equal parts of absolute alcohol and ether, and stained with Löffler's methylene blue.

Leishman's stain (0·15 per cent. in the purest methyl alcohol) is used for staining the malarial parasite. Blood-films, *unfixed*, are flooded with a few drops of the stain, which is spread by tilting, no attempt being made to check evaporation. After half a minute about double the quantity of distilled water is added, allowed to mix with the stain on the film, and staining is continued for five, or in some cases for ten, minutes. The film is then washed in distilled water, some of the water allowed to remain on the film for one minute, and it is then dried and mounted.

Jenner's blood-stain may be used. After the film has dried spontaneously, without fixing it is placed in a pot of the stain for five minutes. The film is washed in distilled water (already coloured with a drop or two of stain) till pink.

Hæmatoxylin and eosin is also a good stain, and very permanent.

The Coccidia.

The coccidia are sporozoa possessing thick cuticles. While some species produce fatal disease in sheep, game birds, and poultry, human infections are rare. The liver and intestines are sites for their activity, and the parasite occurs in large numbers in the droppings.

Coccidium oviforme infects the epithelium of the intestines and bile-ducts in young rabbits with fatal results. In the liver it occurs as a large (30 μ to 40 μ) oval, encapsuled organism in yellowish nodules, consisting of an adenomatous proliferation of the biliary epithelium.

The Piroplasmata.

Piroplasma bigeminum, the cause of Texas fever or bovine malaria, develops in the red blood-corpuscles, first as a minute double body like a diplococcus, which ultimately grows into two pear-shaped bodies. The disease is transmitted through the ticks (*Rhipicephalus annulatus*) which live on the beasts. The female ticks feed on infected animals, and after impregnation fall off and lay their eggs, from which the young ticks develop. The infection passes from the mother through the egg to the young tick, which then infects the animal on which it feeds. It is probable that the parasite passes through a developmental cycle in the ticks. Cattle indigenous to the district are immune.

Piroplasma parvum is found in Rhodesia (red-water) fever of cattle. Prophylactic vaccination is successful.

Amakebe, a disease affecting calves in Uganda, has as the chief symptom the swelling of the lymphatic glands. After recovery from the disease a calf is immune for life. In addition to *P. bigeminum* and *P. mutans*, to which diseases Uganda cattle are immune, the blood contains a small piroplasm indistinguishable from *Piroplasma parvum*, and the Blue Bodies of Koch are found in the spleen. Rhipicephali are carriers of the disease (*R.A.M.C. Journal*, May, 1910).

Piroplasmosis of dogs, horses, and sheep are caused respectively by *P. canis*, *P. equi*, and *P. ovis*.

The Microsporidia.

Nosema apis causes the 'Isle of Wight' bee disease (microsporidiosis). Certain bees act as parasite 'carriers.'

Nosema bombycis causes pébrine in silkworms. It occurs as small glistening, roundish corpuscles, in the caterpillar, butterfly, and egg.

For further information on protozoan diseases see Castellani and Chalmers' 'Manual of Tropical Medicine' Baillière, Tindall and Cox (1913).

CHAPTER XVIII

FERMENTATION—ENZYMES—SULPHUR AND IRON BACTERIA

Fermentation.

FERMENTATION by yeasts, see p. 162 *et seq.* Fermentation by moulds, see p. 170. Bacterial fermentation may involve oxidation, hydration, reduction, or simple decomposition processes.

Acetic Acid Bacteria.—*Mycoderma aceti*, the oxidising agent used in the manufacture of vinegar, consists of two species of bacteria—*B. aceti* (*Hansen*) and *B. Pastorianum* (*Hansen*).

B. aceti (*Kützing*) *Hansen* forms a gelatinous film on 'double' beer when grown at 34° C. for twenty-four hours. The film consists of chains of hour-glass-shaped individuals. The mucilage surrounding the organisms is not stained by iodine. On wort-gelatin at 25° C. grey, waxy, well-defined colonies are formed in four days, but no chains are produced.

B. Pastorianum (*Hansen*) forms a dry, wrinkled film on 'double' beer in twenty-four hours at 34° C. The mucilage is stained blue by iodine. The growth on wort-gelatin consists chiefly of chains.

B. Kützingianum (*Hansen*) on 'double' beer at 34° C. in twenty-four hours forms a dry film, which, unlike *B. Pastorianum*, grows high above the level of the liquid, along the sides of the flask. The mucilage stains blue with iodine, and long chains are seldom found in the colonies on wort-gelatin.

The term 'acetic acid bacteria' is confined to those that give off acetic acid as a main product. Their number is small, though many bacteria produce minute quantities of this acid.

Micrococcus ureæ produces an enzyme (urease) that hydrolyses urea to ammonium carbonate. The cocci vary from 0·8 μ to 1·0 μ in diameter, occur as pairs, tetrads, or chains, and sometimes assume a bacillary form.

B. acidi lactici is aërobic, non-motile, and non-sporing.

The bacillus is 1 μ to 1·7 μ long by about 0·3 μ to 0·4 μ broad, generally occurring in pairs and in strings of four elements. (See also p. 108). It converts milk sugar into lactic acid. Fermentation ceases after a certain amount of lactic acid has been formed, but will recommence if the liquid be neutralised with calcium carbonate. Fermentation will also recommence if the milk is still further left to itself, for moulds neutralise acid.

The **Massol bacillus** (*B. Bulgaricus*), sometimes in conjunction with *Streptococcus lebenis** and a yeast, is used for the production of ' Bulgarian Soured Milk,' for use in diseases arising from auto-intestinal intoxication due to noxious bacterial growth. When acclimatised in the bowel this organism hinders multiplication of other organisms, and, *ipso facto*, the circulation of their metabolic products. It is of prime importance that the milk used should be sterile before inoculation with the Massol culture, and many makers remove more or less of the fat to produce a better-looking product. Only quick-growing strains should be used for the purpose, such as will curdle milk in ten hours. Cultures of the organism are also administered *per os* in the form of tablets or bonbons. Some preparations are useless.

Young organisms are Gram-positive; older ones may be Gram-negative, or only partially retain the stain. Growth is difficult on most media, but good in milk, Cohendy's milk-serum medium, and on milk peptone agar. Growth is said to be best at 105° to 108° F. Currie says the bacilli of *B. Bulgaricus* type in human fæces and human saliva are identical. Some strains produce small amounts of succinic acid, which may account for the presence of this acid in Cheddar cheese.

B. bifidus (Teisser) and *B. acidophilus* (Moro) (see p. 92) are other lactose-fermenters that grow in acid media.

Saccharobacillus Pastorianus is the cause of the ' turning ' of beer, producing lactic acid.

B. butyricus is an aërobic, short or long thin rod, with rounded ends, which forms spores, but seldom threads. It is actively motile, and rapidly liquefies gelatin, with formation of a pellicle. It coagulates and decomposes

* Sewerin considers *S. lebenis* to be identical with *B Bulgaricus*, but his view is not generally accepted.

the albumin of milk, forming peptones and ammonium butyrate.

Clostridium butyricum (*B. amylobacter*) is a long, thick, motile, anaërobic rod, often forming chains. A terminal spore is formed. Butyric acid is formed in solutions of sugars, lactates, and in cellulose-containing plants. The young organisms of the form described by Prazmowski contain granulose, which stains blue with iodine. (See also p. 87.)

Greasiness in Cider.—Kayser finds this disease to be caused by an anaërobic bacillus which ferments sucrose with production of carbon dioxide, alcohol, lactic and acetic acids, also mannitol and lævulose.

Fermentation of Cream.—'Natural starters' for ripening cream naturally contain a mixture of organisms, those producing lactic acid being in greatest number. It is now commonly the practice to use an artificial starter after a preliminary Pasteurisation. In some cases the organism employed appears to be *Streptococcus lacticus*. Similarly, cultures may be added to the cream to produce a good aroma in the butter.

Butter is liable to certain bacterial diseases: turnip flavour (*B. fœtidus lactis*), bitter butter (*B. fluorescens liquefaciens*, *Oidium lactis*, and other organisms), etc.

Bacteria in Cheese Manufacture.—Conn considers that two moulds are necessary for the production of Camembert cheese—a white *Penicillium* (*candidum* ?), and *Oidium lactis* (p. 170). Slimy whey, containing *Streptococcus Hollandicus* (see p. 219), is used as a starter for Edam cheese. *Penicillium glaucum* is the chief agent employed in the ripening of Roquefort, Stilton, and Gorgonzola cheeses. Moulds also play an important part in the production of Brie and other soft cheeses; the growth of certain organisms is encouraged upon the surface of these cheeses, so that the special ferment which they produce can penetrate the body of the cheese and bring about certain characteristic changes.

Tobacco-Curing.—The leaves are suspended in barns, when the leaf enzymes convert the starch into sugar. The browning of the leaves which takes place during this period is a complicated process, and is the outward expression of a large number of very little understood processes which are not bacterial. The dried tobacco-leaves are

stacked to undergo heating, and fermentation occurs, possibly by the action of members of the proteus, subtilis, and mycoides groups, or by one of the *Bacilli tobacci*, but the main trend of opinion is to regard the process as due to oxidising enzymes.

Tanning.—Hides may be freed from hair by treatment with sulphites or milk of lime, or by allowing hides to putrefy. This is followed by a 'pickle' of bran and canine or avian excreta. Lactic acid is developed, which combines with the lime to form a soluble lactate. Gas-forming bacteria (*B. furfuri* is the only one yet isolated) grow and cause the hides to swell. The changes that take place in the next stage, in the tan-pit, are due to bacteria; the process is essentially a souring process, in which success lies in the proper regulation of the amount of lactic acid produced.

Retting of flax appears to be due to the growth of bacteria, one of which has been isolated by Winogradsky, and is used for the work as a pure culture.

Fermentation processes are involved in the preparation of tea, coffee, cocoa, and indigo. On the Continent low-grade wines are improved by the use of pure cultures of bacteria obtained from high-class vintages, whereby much of the characteristic aroma and bouquet of the latter are communicated to the wine.

The Enzymes.

The enzymes are unorganised ferments, often secreted by bacteria and the cells of animals and plants. In many cases (groups 1, 2, 3, 5 and 6) they act by hydrolysis, or, more correctly, by zymolysis. The most important groups are the following:

1. *Proteolytic*, changing proteins into proteoses, peptones, and further products of hydrolysis, as in the case of pyocyanase, trypsin, and pepsin.

2. *Amylolytic*, converting amyloses (starch, glycogen) into sugars, such as diastase, ptyalin, and amylopsin.

3. *Inversive*, which convert disaccharides (lactose, maltose, cane-sugar) into glucose. Invertase, which occurs in the cells of yeasts (p. 162) and in the intestinal juice, is an example of this class.

4. *Coagulative*, converting soluble into insoluble proteins, such as rennet (chymosin), the fibrin and myosin ferments.

5. *Steatolytic*, splitting fats into fatty acids and glycerin. Lipase (steapsin), which occurs in the pancreatic juice and in many plants, is an example of this class.

6. *Peptolytic*, splitting proteoses and peptones into polypeptides and amino-acids.

Other groups bear oxygen and produce oxidation (Oxydases), produce reduction (Reductases), or break off amino-groups from amino-compounds (Deamidases).

While some enzymes can act alone, others require the stimulus of an activating agent or co-enzyme (p. 163). Antecedent substances (zymogens) which produce the enzyme exist in the cells. While an enzyme will only act under specific conditions on a specific substance, its power in this limited sphere is inexhaustible, as is that of an inorganic catalyst. Enzyme action is generally reversible, and during an analytic or splitting reaction there goes on at the same time a synthesis reproducing the original substrate.

Tissue enzymes may be defensive; the blood of a pregnant woman elaborates a specific defensive enzyme in response to the entry of placental proteins into her own blood-stream.

The Sulphur Bacteria.

David Ellis classes under this heading those organisms which have the power of assimilating sulphuretted hydrogen and effecting its oxidation: the *Beggiatoa* (see p. 160); the *Thiothrix*, which differ from the *Beggiatoa* in being non-motile, and having a common thread membrane; the *Thiophysa*, which form no threads; and the *Purpur bacteria*, which contain a colouring matter in the cells, *bacterio-purpurin*, which seems to have a function in abstracting oxygen from the medium.

The Iron Bacteria.

These organisms occur in ferruginous waters, and convert the soluble bicarbonate, $FeH_2(CO_3)_2$, into ferric hydroxide, $Fe_2(OH)_6$, the organisms becoming coated with the latter. In addition to *Crenothrix polyspora* and *Cladothrix dichotoma* (p. 160), the following organisms act in the manner indicated:

Leptothrix ochracea (*Chlamydothrix ochracea*) averages in length from 100 μ to 120 μ. Reproduction takes place

by fission or by formation of conidia. The organism takes the form of a straight cylindrical rod.

Gallionella ferruginea (*Chlamydothrix ferruginea*) assumes the shape of a hairpin, with the prongs twisted round one another.

Spirophyllum ferrugineum (Ellis) is a flat, tape-like organism, spirally twisted, which multiplies by conidia formation.

These higher bacteria have not the power to dehydrate and reduce the ferric hydroxide to bog ore. 'Bacillus M. 7' (Mumford) not only precipitates ferric hydroxide from iron solutions, but, by an anaërobic action, also transforms this to bog ore with partial reduction of the iron to a ferrous state. Mumford thinks that to this organism are probably due the deposits of bog ore. 'Bacillus M. 7' is a motile, sporulating bacillus that occurs singly or in chains of three or four units.

For further information on the iron and sulphur bacteria, and also for the bacteriology of fermentation processes in the arts, see David Ellis's 'Outlines of Bacteriology.'

BACTERIAL DISEASES OF PLANTS.

Black Rot of Cabbage (*Pseudomonas* or *B. campestris*) is a disease of the fibro-vascular bundles, which become dark brown or black. On cutting across the petiole of a diseased leaf the affected bundles are seen as dark points. When so many of the bundles are affected as to cut off the supply of water to a leaf, the blade dries up and resembles a piece of brown parchment, the blackened veinlets standing out sharply against the brown background. As the leaves in the head become affected they decay, producing a dark vile-smelling mass.

Commercial seed is a factor in the distribution of the disease. The organism may gain entrance through broken roots at the time of transplanting, or through the water pores at the margin of the leaves. All cultivated plants of the Crucifer family are liable to be attacked. Slugs and insects may convey the disease. *B. campestris* is a short, motile, Gram-negative bacillus, which liquefies gelatin, digests casein, and produces a yellow pigment on potato.

B. solanacearum affects members of the *Solanaceæ* (tomato, egg-plant, and potato), producing discoloration of shoots, stem, and leaves (brown rot). The fruit and tubers are attacked also. *B. solanacearum* rots the skins, and then, assisted by other bacteria and fungi, rots the centres too. The Colorado potato beetle is sometimes the traumatic agent, and can spread the disease. Cucurbitaceous plants are immune. The organism grows on ordinary culture media, forming a yellowish-brown pigment.

B. tracheiphilus produces a wilting of the leaves (wilt disease) in cucumbers, pumpkins, and other cucurbitaceous plants, but does not affect solanaceous ones It forms a white viscid growth on agar, does not affect milk, and produces acid in glucose and saccharose.

B. amylovorus produces browning and death (pear blight) in the twigs of pear-trees. Peritrichally-arranged flagella are present, and the organism is motile. A turbidity is produced in broth, with formation of a pellicle. Transmission is effected by bees.

B. oleæ produces nodular formations on the olive (olive-knot disease). *B. hyacinthi* causes the yellow disease of hyacinths.

Pseudomonas destructans, a short rod, with a single terminal flagellum, causes ' white rot ' in turnips. Several bacterial diseases occur in the cultivated potato—viz., *Micrococcus nuclei*, *M. imperatoris*, *M. pellucidus*, always found associated with the ' scab,' *M. albidus* and *M. flavidus*.

Penicillium glaucum most commonly causes the rotting of fruits. In apples and pears this is accompanied by *Mucor pyriformis*, and in the case of medlars the latter is much the most common fungus. In lemons, oranges, and other tropical and subtropical fruits, *P. glaucum* is associated with two other closely-allied species, *P. italicum* and *olivaceum*. In plums *Mucor racemosus* has been observed. In grapes *P. glaucum* and *Botrytis cinerea* are the most common fungi. The latter species forms the grey tufts on walnuts.

B. tumefaciens is the primary cause of many of the tumour-like growths on the roots of plants (crown gall).

Gum-producing Bacteria.—Greig Smith and Edie have practically established as a fact the bacterial origin of

gum. The resistance of trees requires to be lowered by fire or mechanical injury before the bacteria can work, and it is thought that ants carry the gum-producing organisms from tree to tree.

For further information on phytopathology, see Jakob Eriksson's 'Fungoid Diseases of Agricultural Plants' (Baillière, Tindall and Cox, 1912).

CHAPTER XIX

DISEASES OF QUESTIONABLE ORIGIN

Scarlet Fever.—*Streptococcus scarlatinæ* (Klein), or *S. conglomeratus*, occurs in desquamating particles of skin, blood, sputa, tissues, urine, and mucous secretion of the throat of patients (see p. 134). Mallory has found small rounded bodies resembling protozoa, which others regard as degenerate leucocytes. They sometimes show rosette forms and stain with methylene blue. Jordan regards them as characteristic of the disease. Perhaps, at one stage at any rate, the virus is filterable.

The disease is more prevalent in the northern part of Europe than in other parts of the Continent. Epidemics exhibit a quinquennial recurrence, the mortality being about 3 per cent., and greatest at the age of five. One attack is usually protective.

Formerly the chief danger of dissemination was thought to lie in the desquamating particles of skin, but the faucial, nasal, and aural secretions are now regarded as the more important sources of infection. Milk is a frequent medium for the conveyance of the disease. Much difference of opinion exists as to whether the disease can arise from a bovine source.

Malignant Disease.—Hitherto, although a large number of organisms have been described, none has been shown to produce either carcinoma or sarcoma. According to Rous, the ætiological agent of a spindle-celled sarcoma in chickens proved to be a filterable virus.

Epilepsy.—Reed thinks this disease is an infection with a gas-producing bacillus.

Pellagra.—Sambon believes this to be of protozoan

origin, and to be transmitted by a biting sandfly of the genus *Simulium* which haunts running streams.

Rheumatic Fever is perhaps an acute specific disease (see also p. 133). Various micro-organisms have been isolated, particularly staphylococci and streptococci, and a large bacillus (by Achalme) resembling the *B. Welchii*. Hewlett believes the bacillus of Achalme to be identical with *B. Welchii*, and says it is probably a terminal infection or a contamination.

Cholera Nostras, or English Cholera.—Klein found the colon bacillus and *Proteus vulgaris* to be abundant in the dejecta of patients, and the disease has been attributed to abnormal putrefaction by these and other organisms in the bowel.

Summer Diarrhœa of Infants.—Booker was unable to identify any specific organism, but found the colon bacillus, *Proteus vulgaris*, and streptococci very abundant. *B. dysenteriæ* is present in a large number of cases. The symptoms of tyrotoxicon poisoning resemble those of cholera infantum. Vaughan, who discovered this body, also obtained toxic bodies from cultures of Booker's bacteria, which produced vomiting, purging, and sometimes death in dogs. Sidney Martin has shown that the products of *Proteus vulgaris* injected into rabbits cause depression of temperature and watery evacuations.

Schölberg and Mackenzie Wallis (L.G.B. Med. Off. Rep., 1911) found peptones and peptone-like substances of toxic nature in milk in the summer, and in milk that had been incubated at 37° C. for fifteen to twenty-four hours. These substances arise from bacterial activity, and are thought to have some relation to the epidemic diarrhœa of infants.

See also *Saccharomyces ruber* (p. 218) and Morgan's No. 1 bacillus (p. 97).

Louping Ill.—The cause of this disease, which is common among Scotch sheep, is unknown. The disease is transmitted by a tick.

Hay Fever.—Mouneyrat thinks that hay fever is an infection provoked by an unknown micro-organism transported at certain times of the year by dust of the pollen of flowers.

THE FILTERABLE VIRUSES.

Some diseases are produced by organisms so minute that they will pass through the pores of a kieselguhr or porcelain filter, and remain invisible under the microscope (ultra-microscopic organisms). In some cases, the organisms are only ultra-microscopic and filterable at one stage, the filtered virus producing organisms more or less well within the limits of visibility.

Some filterable organisms (those of rabies, variola, vaccinia, and anterior poliomyelitis, for instance) will live in glycerin for weeks without losing their virulence. According to Cockayne (*Medical Press*, February 12, 1913), some are readily destroyed by weak antiseptics such as 2 per cent. phenol, menthol, or hydrogen peroxide. Some resist very low temperature: $-2°$ C. to $-12°$ C. Many are readily killed by exposure to 50° to 65° C. for a few minutes. With the exception of the virus of cattle pleuro-pneumonia and the possible exceptions of those of infantile paralysis and typhus, none has been cultured on artificial media (see also Twort, *Lancet*, December 4, 1915).

Cockayne (*loc. cit.*) says: 'In those filterable organisms which can only pass through a kieselguhr filter, and are therefore visible under the microscope, there is a resemblance in staining reactions so far as these have been carried out. They stain well with Löffler's methylene blue, less well with hæmatoxylin, and there is round them a clear halo—an appearance altogether different from that obtained by controls of crushed liver pulp passed through a similar filter.'

Pleuro-pneumonia in cattle is characterised by the peculiar marbled appearance of the lungs, accompanied by great distension of the connective tissue, with a yellowish albuminous fluid. The serious symptoms are due to toxins, and death frequently ensues. Roux and Nocard describe a minute refractile body just visible with the highest and best powers, but small enough to pass through the pores of a Berkefeld filter. It was found to stain with gentian violet, methyl violet, and hot carbol fuchsin, though never more than half the particles took up the stain.

Hydrophobia (Rabies).—Infection in man and animals takes place as a result of bites from dogs, cats, wolves, jackals, cattle, or other animals. The virus is present in the saliva and in the central nervous system. The incubation period in a dog is from about three to eight weeks, though it may extend to six months. In man the disease usually develops in from eight days to six weeks, up to perhaps two years. In the majority of cases it is in the neighbourhood of ten weeks.

Negri bodies, varying from small structureless round bodies to larger ones, which may be round or ovoid and show a circle of chromatoid granules round a central granule, are constantly present, and afford a means of diagnosis. The virus is probably ultra-microscopic. The Pasteur method of treatment commences with inoculation of the spinal cords of rabbits which have been attenuated by suspension in a drying chamber for fourteen days, and then made into an emulsion. Virus of gradually increasing strength is injected until a much more virulent emulsion from a cord that has only been dried for three days is tolerated, which, without previous treatment, would be dangerous. Such is the success of these antirabic inoculations, that among the persons treated (over 1,000) at the Pasteur Institute, during 1912, 1913 and 1914, no death occurred. The immune serum of a protected animal is said to have a marked curative effect.

Swine Fever (see also p. 96).—Swine fever may be disseminated by the excreta of infected pigs or by contact with infected pigs. Stockman mentions that pigs which to all appearance had recovered from the disease may, in a small proportion of cases, be capable of spreading infection for months. The Departmental Committee on Swine Fever found that rats are not pathological carriers of the disease, nor is the disease propagated by external parasites, and that, while infection may be carried locally by persons, vehicles, or animals in contact with infected pigs, wide dissemination is due to the movement of pigs.

The Board of Agriculture considers that, if proper precautions are taken, immunity from swine fever can be established by serum treatment. The Committee recommended the immunisation of herds by simultaneous administration of serum and virus. They consider the lapse of a short period of time (a fortnight is suggested)

may be relied upon for disinfection of premises, and should be regarded as preferable to chemical disinfection in the case of large quantities of manure and of premises not readily capable of being disinfected by artificial means.

Typhus Fever.—Hort (*Medical Press*, October 28, 1914), after filtration of typhus urine through Berkefeld filters, found small cocco-bacillary forms which grew on human blood-agar. He obtained the same organism in cultures from blood and cerebro-spinal fluid, and these cultures produced high continued fever in bonnet monkeys. The small coccal and bacillary forms give place to large coccal and bacillary forms when urine, blood, or cerebro-spinal fluid, is incubated, which forms appear incapable of producing fever in bonnet monkeys. Hort regards the filter-passing cocco-bacillus as infective, and thinks it probably capable of mutation into the larger cocco-bacillary forms. Sambon found the body louse was the transmitting agent of typhus.

Measles.—The virus appears to be filterable. It may lose its infectivity after fifteen minutes at 55° C., and resist freezing for twenty-five hours (Goldberger and Anderson). These workers and others have shown that the blood and the nasal and oral secretions of patients contain the infective agent from twenty-four hours preceding to twenty-four hours following the appearance of the eruption.

Rinderpest (cattle plague) occurs in India and South Africa. The virus is filterable. Sir George Turner stamped out rinderpest in Cape Colony by the simultaneous inoculation with virus and serum.

Foot-and-Mouth Disease, aphthous fever, or *eczema epizoötica*, is an infectious disease of cattle, characterised by a vesicular eruption of the mouth, teats, and about the feet. It affects also sheep and pigs, and may be communicated to man by milk or cheese and butter made from it, and usually produces in the human subject an aphthous condition of the mouth. The organism is filterable through a Berkefeld filter. The blood is infective only in the earliest stage of the disease. The virus is present in the contents of the vesicles which contaminate the saliva, litter, milk, etc. The virus is very susceptible to desiccation and light, is killed by a temperature of 56° to 70° C., but under natural conditions may remain active for months. Horses, dogs, and cats occasionally

contract the disease, and with birds, rats, and fowls, may mechanically carry infection.

Canine Distemper.—*Bacillus bronchisepticus* (M'Gowan) is described as present in the respiratory tract in distemper. In culture a powerful nerve toxin is produced, and the disease simulates infantile paralysis of man. Some cases of distemper, at any rate, appear to have a filterable virus.

Dikkop (blue tongue or horse sickness) is prevalent up the Blue Nile. The virus is ultra-visible, and appears to be conveyed by mosquitoes (Balfour).

Sheep-pox.—Cockayne says the organism passes through a Berkefeld, but never through the Chamberland F.

Yellow Fever.—Sanarelli's *B. icteroides* (p. 96), previously supposed to cause this disease, is now regarded as a casual invader. The virus proves to be filterable, ultramicroscopic, and probably a protozoon. A species of mosquito—*Stegomyia fasciata* (*calopus*)—is the transmitting agent, and cannot convey the infection until twelve days after biting a fever patient. The organism must have a life cycle in the mosquito as well as one in man. Research is in progress upon the relationship of the so-called 'Seidelin bodies' to the virus. Prophylactic measures, aiming at the destruction of the insects and larvæ, have proved successful in the highest degree.

Dengue.—Ashburn and Craig passed dengue blood through a porcelain filter, and with the filtrate produced the disease in healthy men after an incubation period of three and a half days. The disease is conveyed by a mosquito, *Culex fatigens*, Wiedemann, which, after biting an infected person, is not capable of conveying the disease until a week has elapsed.

Phlebotomus (Sandfly) Fever.—The virus passes Berkefeld and Chamberland F. filters. It is conveyed by a sandfly, *Phlebotomus pappatasii*, which is not infective until a week after biting.

Mumps.—Cocci have been found in this disease by many authors, but Gordon found that in a proportion of cases a virus that passes through a Berkefeld filter occurs in the saliva.

Variola and Vaccinia (Smallpox and Cowpox).—Opinions as to the relationship of these diseases are controversial, the tendency being to regard vaccinia as a modified form of variola. No specific organism can be distinctly

connected with either disease, although many organisms have been isolated, notably *Bacillus albus variolæ* of Klein and Copeman, and what is believed to be a protozoon, the *Cytoryctes variolæ*. It seems probable that the viruses of both diseases are filterable.

Acute Anterior Poliomyelitis (Infantile Paralysis).—Most cases of this disease (which may occur in a sporadic and also in an epidemic form) affect children, principally in the second and third years of life. The epidemic type may attack older children and adults. The virus is filterable, and for many months in pure glycerin keeps without loss of virulence.

Flexner and Lewis have shown that the virus can enter, and is eliminated by, the mucosa of the naso-pharynx. Transmission may take place by 'carriers' who themselves remain healthy. The virus is carried by the house-fly both on the surface of its body and within its gastro-intestinal tract. It can also be transmitted by bed-bugs and stable flies, but not, as far as is known, by fleas or mosquitoes. The virus remained potent in sterile milk and sterile water for at least thirty-one days, and was found in a patient seven months after the onset of the disease. Zappert found it in the dust of a patient's bedroom. It is destroyed at 45° to 50° C. in thirty minutes. The virus of infantile paralysis has not been definitely cultivated, but Flexner and Noguchi thought it to multiply in a medium of broth *cum* human serum under anaërobic conditions. Diseases similar to poliomyelitis have been described in horses, cows, pigs, chickens, and dogs. As it is impossible, apparently, to infect these animals with the human virus, the diseases are probably not identical with the human one. Alexander suggests that the virus of poliomyelitis may remain in the water and slime of public baths. In one epidemic over 50 per cent. of one series of cases had been swimming or wading in water contaminated by sewage.

Epidemic Nephritis.—Langdon Brown (*R.A.M.C. Journ.*, July, 1915) says bacteria could apparently be excluded as the infective agent in the acute nephritis that occurred among the soldiers of the British Expeditionary Force in France, but a filter-passing ultra-microscopic organism could not be excluded. The point of entrance of the infection was possibly the tonsils.

In addition to diseases already mentioned, Cockayne gives the following list of diseases proved or suspected on strong grounds to be due to a filterable virus: African horse sickness and the nearly allied catarrhal fever of sheep (both S. African). Myxomatosis of rabbits (also affecting dog and man, S. America). Agalasie contagieuse de brebis. Infectious or pernicious anæmia of the horse. Trachoma and a non-gonococcal urethritis probably due to the same organism. Epithelioma contagiosum of fowls and diphtheria of wood-pigeons, probably forms of the same disease. 'Farcin crytococcique.' A disease (jaunisse) of silkworms. Mosaic disease of the tobacco plant. Variola of carp. Disease of the lips of barbel. Cyanolophia gallinarum, or chicken typhus, and an allied disease affecting three species of thrush and the starling in Italy.

CHAPTER XX

THE BACTERIOLOGY OF SEWAGE, SHELLFISH, MEAT, SOIL, AIR, AND MILK

The Bacteriology of Sewage.

The number of bacteria in ordinary town sewage varies considerably, according to the age of the sewage, the season, temperature, locality, and sometimes with the nature of the waste liquors from factories. The number often reaches several million per cubic centimetre. The number of *B. coli* is given by Klein and Houston as from 90,000 to 2,000,000 per cubic centimetre, 100,000 being an average; of *B. Welchii*, as from 100 to 1,000 per cubic centimetre; and of streptococci, as at least 1,000 per c.c.

In addition to the above and those mentioned seriatim at the end of the monograph, *B. pyocyaneus*, *B. mycoides*, *B. subflavus*, *B. cloacæ*, staphylococci, *Cladothrix dichotoma*, *Beggiatoa alba*, *B. fluorescens stercolatus*, *B. filamentosus*, *M. ureæ*, and many others, are present. Researches on the longevity of the typhoid bacillus in sewage point to the fact that in crude sewage it cannot be expected to survive longer than a fortnight, while the cholera vibrio, on the other hand, was found by Houston to survive for a month in one instance.

The following scheme (adapted by Moor and Hewlett, after Houston) is applicable to sewage and effluents. A fair average sample of the sewage or effluent must be obtained by mixing portions obtained at intervals. The mixture should be strained through muslin.

Tests.	*Procedure.*	*Amount of Sewage in c.c.*
1. Total number of bacteria	Gelatin and agar plate cultivations	0·001, 0·0001, 0·00001
2. Number of spores of aërobes	Gelatin plate cultures with material previously heated to 80° C. for ten minutes	1·0, 0·1, 0·01
3. Number of spores of anaërobes	Agar plate cultures with material previously heated to 80° C. for ten minutes and incubated anaërobically	1·0, 0·1, 0·01
4. Number of organisms liquefying gelatin	*Surface* gelatin plates	0·001, 0·0001, 0·00001
5. Spores of *B. Welchii*	Milk cultures (see p. 87)	0·1, 0·01, 0·001
6. Number of *B. coli*	*Surface* Conradi-Drigalski plates, or bile salt broth	0·001, 0·0001, 0·00001
7. Number of streptococci	*Surface* plates of Conradi-Drigalski medium	0·01, 0·001, 0·0001

In addition, effluents should be submitted to the two following tests:

1. Incubate some in beakers at 22° C. and 37° C. for some days. A good effluent should yield little or no unpleasant odour (an unpleasant odour indicates the

presence of decomposable organic matter, and such an effluent might give rise to a nuisance).

2. Place a goldfish or two in a bowl of the effluent. The fish will live in, and be unaffected by, a satisfactory effluent. In the eyes of the law a fish is an animal, and this test may only be performed by a licensee under the Vivisection Act.

An effluent of sewage treated by land filtration should show a considerable reduction in the total number of bacteria, and in the number of *B. coli*, compared with the original sewage; but an effluent from a bacterial system of treatment may contain as many bacteria as, or actually more bacteria than, the original sewage.

Apart from the nitrifying organisms, very little of practical value is known of the bacteria concerned in sewage treatment, and their ability. Especially is this true of those acting in septic tanks and contact beds. With further enlightenment there will be fewer failures.

Houston, in his capacity of bateriologist to the Royal Commission on Sewage Disposal, while recommending as 'counsel of perfection' the 'complete sterilisation of sewage effluents' in the case of drinking-water streams, suggested as a 'practicable standard' 'partial sterilisation (absence of *B. coli* from 1 c.c. of the effluent).' Houston also suggested 'provisional' standards to apply to sewage effluents for non-drinking-water streams, as follows:

Total number { Gelatin at 20° C., less than 100,000 per c.c.
of bacteria { Agar at 37° C., less than 10,000 per c.c.

B. coli, less than 1,000 per c.c.

B. enteritidis sporogenes test .. .. .. } Negative results
'Gas' test (twenty-four hours at 20° C.) .. } with 0·1 c.c.

Indol test (five days at 37° C.) .. .. ..
Neutral red broth test (two days at 37° C.) .. } Negative results
Bile-salt broth test (two days at 37° C.) .. } with 0·001 c.c.
Litmus milk (modified) test (two days at 37° C.)

These are primary standards; Houston's secondary standards are arrived at by rendering the primary standards ten times more lenient. He does not suggest these standards in an administrative sense; they are merely arbitrary, and designed solely for comparative purposes.

Prescott and Winslow think even the above primary standard is far too lenient, and argue that 'either organic

purity alone is necessary, as at many sewage disposal plants, or a higher grade of purity than this (the primary standard) should be attained.'

The Royal Commission deprecate the adoption of standards of bacteriological purity or of sterilisation processes, both on the ground of the serious additional cost which these would entail, and of the 'false sense of security' which the adoption of such measures would be apt to engender.

Some Organisms met with in Sewage.—The granular or sewage variety of *Proteus Zenkeri* is a non-liquefying, non-sporing, aërobic, non-motile bacillus, and occurs in the form of short and long chains and filaments. It grows well in phenolated gelatin and phenolated broth, does not form gas in a gelatin-shake culture, does not coagulate milk, and gives no indole in broth after three days' growth.

P. vulgaris, P. mirabilis, P. Zenkeri, are found in putrefying substances, sewage, and water. The first two are motile, produce involution forms, liquefy gelatin and blood-serum, and give off a decided putrefactive odour from cultures. *P. Zenkeri* is non-motile, does not liquefy gelatin or blood-serum, and the putrefactive odour is absent from the cultures. All three species produce local abscesses and symptoms of toxæmia when injected into small animals.

Herter and Broeck (*Jour. Biol. Chem.*, 1911, 491) say that *P. vulgaris* ferments dextrose and sucrose, but not lactose; it destroys some native proteins, producing ammonia, primary amines, hydrogen sulphide, fatty acids of high molecular weight, aromatic hydroxy-acids, indole, and indole-acetic acid. They also obtained thermostable toxic material from the bacteria. *P. vulgaris* was formerly called *Bacterium termo.* The varieties of proteus mentioned above are now generally referred to as *Bacillus proteus.*

B. fluorescens liquefaciens, B. fluorescens non-liquefaciens.—These water organisms are short Gram-negative bacilli. The former liquefies gelatin, and is motile; the latter does not liquefy gelatin, and is non-motile. Gelatin streaks show a fluorescent green, and the growth on potatoes is brown in both cases (see p. 130).

B. subtilis (*hay bacillus*) occurs in hay, air, water, fæces, etc. It is about 2 μ to 3 μ long by 1 μ broad, about the

same length as, but somewhat narrower than, the anthrax bacillus. It has rounded ends, grows into long threads, and is very motile, having long flagella; forms ovoid spores about 1·2 μ long by 0·6 μ broad, which germinate at right angles to the long diameter, and are very resistant to heat, surviving dry heat of 120° C. for one hour (see p. 9). The bacillus is strictly aërobic and Gram-positive. The colonies on gelatin plates become visible in about two days, as small white dots in the depth, whereas on the surface they show small greyish liquefied circles. In a gelatin stab a liquefied funnel-shaped depression forms, the lower part throwing out lateral feathery extensions. The whole of the gelatin is soon liquefied, and a tough pellicle forms on the surface, and a quantity of flocculent matter collects at the bottom of the tube. A white, opaque, moist expansion is formed on agar, which afterwards becomes dry and furrowed; a uniform turbidity with pellicle develops in broth, and a moist cream-like expansion, dull and never shining, forms over the whole surface of potato.

B. ramosus (*wurzel bacillus*) strongly reduces nitrates to nitrites. The bacillus is Gram-positive, about 7 μ long and 1·7 μ broad, with rounded ends. It occurs in long threads, and has resistant spores. On gelatin plates cloudy centres, with root-like branches extending in every direction, are seen; the gelatin is slowly liquefied. In gelatin stabs a slight depression is seen after the second day, whilst the needle track in the depth has a greyish woolly appearance. The whole contents of the tube then becomes liquid, a tough pellicle forming on the surface. It grows on carbolated media. It grows rapidly over the whole surface of agar; in the depth is seen the characteristic woolly appearance. A white dry expansion is formed on potato.

B. mesentericus fuscus and *B. mesentericus vulgatus* ('potato bacillus') are found on vegetables and in water. They are short, motile, sporulating, Gram-positive organisms, which liquefy gelatin. *Fuscus* forms a yellowish-brown growth on agar, while *vulgatus* gives a dirty white growth. Red (*ruber*) and black (*niger*) varieties are also known.

B. tholoeideum.—An intestinal organism invariably found in sewage. It produces a septicæmia in mice and

guinea-pigs, and gives yellowish growths on potato and gelatin streaks.

Hydrogen-Sulphide-forming Bacteria. — Fæces, both human and animal, contain bacteria capable of producing hydrogen sulphide from peptone water. Chamot and Redfield (abst. *Analyst*, 1915, 351) found this group do not actively ferment carbohydrates.

The Bacteriology of Shellfish.

Epidemics of typhoid have arisen through the ingestion of bivalves and molluscs obtained from beds polluted with sewage containing typhoid bacilli (see p. 100). Oysters are examined for sewage pollution by Houston's method. Ten oysters are taken from a sample, thoroughly cleansed by scrubbing in tap-water and rinsing in sterile water. They are opened aseptically, and the fish is minced and added, together with any fluid contained in the shell, to sterile water in a sterile graduated cylinder, and the bulk is made up to a litre with sterile water. Each 100 c.c. of the emulsion represents one fish. With the liquor thus obtained cultures are made in—

(*a*) Litmus lactose bile-salt peptone-water for detecting *B. coli*, amounts being taken varying from 100 c.c. to 0·0001 c.c., representing quantities of the fish from one fish to one-millionth of a fish.

(*b*) Sterile milk for detecting *B. Welchii*, amounts being taken of from 10 c.c. to 0·001 c.c., representing quantities of fish from one-tenth of a fish to one-hundred-thousandth of a fish.

Agar and gelatin plates are also made for enumeration.

Houston's standards are—To reject oysters containing 1,000 (lenient standard) or 100 (stringent standard) *B. coli*, or 100 (lenient standard) or 10 (stringent standard) spores of *B. Welchii*, respectively per oyster.

On the other hand, Hewlett, finding that oysters from pure layings contain no *B. coli*, is of opinion that even ten *B. coli* per fish should be viewed with suspicion.

Nash is convinced that large numbers of 'indication' organisms will not always justify the conclusion that the shellfish have been exposed to what is generally understood by 'sewage' pollution—that is, human sewage coming down through a sewer outfall. 'Cattle, sheep, and geese are grazed on low-lying marsh-lands, which

at exceptional tides, and sometimes even with ordinary tides, are covered at high-water. These animals (and not least among them the geese) deposit on the marsh-lands a very large amount of excrement, which is washed off the marsh-lands at high-water. Cockles and mussels especially abound in the vicinity of these grazing grounds, which are often intersected by creeks. The sand in these creeks will often be found quite black under the surface, and even the shells of the cockles quite discoloured, and yet there may be no sewer outfall within several miles.'

Letts attributed the excess of *Ulva latissima* in Belfast Lough as being due to its sewage-polluted waters. Nash, however, demonstrated that *Ulva latissima* may be found in creeks or on shores adjacent to extensive tracts of low-lying marsh-lands which are used for grazing purposes, but yet miles from any large sewer outfall. He therefore concluded its presence on mussels to be no indication of a polluted source.

The Bacteriology of Meat.

In addition to examination for trichina, vermes, etc. meat may have to be examined for tuberculosis and other animal diseases which are dealt with under their respective monographs, or for organisms causing food poisoning (see *B. enteritidis*, p. 93, *B. botulinus*, p. 83, *B. paratyphosus* β, p. 95, and *B. suipestifer*, p. 96). A filterable virus, a variant of *B. coli*, *B. proteus*, and *Staphylococcus pyogenes aureus*, have also been described as organisms producing food poisoning. The isolation of proteus from suspected material, even if it gives a positive agglutination, requires cautious consideration before it is connected with the disease, as McWeeney has shown that it tends to undergo auto-agglutination.

Varieties of Phycomyces, Penicillium, Mucor, Verticillium, and Oospora, are the principal moulds that are found on refrigerated meat. 'Red spot' is due to *B. prodigiosus*, and *Cladosporium herbarum*, causing 'black spot,' is frequent. Klein investigated some brown spots on frozen meat, and found a variety of yeast. Experiments with animals showed that the yeast was not harmful. Black spots are sometimes present in Argentine beef on the lower or thin parts of fascia, in the fatty portion of the thick parts, and in the fat belonging to

the inner surface of the flanks. Klein found *Oidium carnis* to be the cause, and states that it is harmless to animals.

B. fœdans (Klein) causes taint in miscured hams, which have undergone ' dry curing ' only. It is a short cylindrical rod producing chains and filaments, and is an obligatory anaërobe, non-motile, Gram-positive, and apparently non-sporing. Growth does not take place on ordinary media, but glucose gelatin, glucose broth, and particularly glucose pork broth, were found suitable. Milk acquired a nauseating odour, but guinea-pigs were unaffected by subcutaneous injection.

Beveridge, Fowler, and Fawcus, describe an organism isolated from blown tins of corned beef, which is apparently identical with *B. cadaveris sporogenes* (Klein) and *B. putrificus coli* (Bienstock). This organism is an obligatory anaërobe, motile, with a slow wavy movement, and forms large terminal spores. In four or five days at blood-heat litmus-milk is decolorised, and sometimes clotted. Later slow digestion of the casein takes place, leaving a clear yellow whey. Digestion is complete in seven to fourteen days, merely leaving a granular deposit. Plentiful gas is formed in neutral red glucose agar, and in all cultures it gives off an exceedingly putrid odour. The organism is apparently non-pathogenic, but decomposes tinned meats and renders them unfit for consumption. The spores are only killed by a temperature of 112° C. in twenty-five minutes, 115° C. in ten minutes, or 117° C. in less than five minutes. Tinned meats often contain sporing organisms of the *B. subtilis* (p. 205) and *mesentericus* (p. 206) groups. Wanhill and Beveridge incubate tins at 37° C. for fourteen days, when bulging indicates the presence of gas-forming bacilli, probably *cadaveris*.

In some cases of tinned meats which have caused food poisoning nothing unusual is noted in the appearance, but in others the gelatin is liquid at ordinary temperatures, or discoloration, sickly odour, or a soapy taste, is noted. For further information see Savage's Report to L.G.B. on Bacterial Food Poisoning and Food Infections, 1913.

As food is still sent to analysts to be examined for ' ptomaines,' the following quotation from a Memorandum

by the Local Government Board (September, 1911) is given as representing the considered opinion of the best authorities on the subject: ' Ordinarily little is to be gained by so doing. It is by no means certain that " ptomaines " in the sense of alkaloidal substances produced by bacterial action are present in meat foods which have caused poisoning, and the significance of the reactions which are held to demonstrate the presence of these substances is a matter of considerable doubt.'

The Bacteriology of Soil.

Surface soil is very rich in bacteria, the number below a depth of 1 metre being small. Fränkel found that the superficial layers of the soil of a fruit orchard contained from 50,000 to 350,000 organisms per gramme. The greatest number was at $\frac{1}{4}$ to $\frac{1}{2}$ metre below the surface. At a depth of from $\frac{3}{4}$ to $1\frac{1}{2}$ metres there was a very abrupt diminution in the number of bacteria. From 200,000 organisms at a depth of $\frac{1}{2}$ metre, the number fell to 2,000 at a depth of 1 metre, from 250,000 at $\frac{3}{4}$ metre to 200 at 1 metre. In virgin soil there is a dividing-line at a depth of from $\frac{3}{4}$ metre to $1\frac{1}{2}$ metres, below which very few bacteria are found, thus showing the ground water region is quite free, or nearly free, from micro-organisms, notwithstanding the vast number upon the surface of the soil.

Houston found surface virgin sandy soil to contain less than 100,000 bacteria per gramme, other virgin soils about 1,000,000, garden soil from 1,000,000 to 2,000,000, and grossly-polluted surface soils up to 115,000,000 bacteria per gramme. Houston regards *B. mycoides* and the Bismarck brown cladothrix as especially characteristic of soil.

James Buchanan Young found the soil of graveyards to be very rich in micro-organisms, particularly those of a liquefying type, *Proteus vulgaris* being present in great numbers. Their action is so effective that he found no notable quantities of organic carbon and nitrogen in the upper layers, and not so very much more in the lower layers than are found in virgin soil. He found, on the whole, that the bodies do not greatly influence the number of micro-organisms found.

Two peat organisms, ' O ' and ' Q ' (Houston), produce

acid when grown in peat-infusion, which has a great solvent action upon lead. It is to these organisms that the plumbo-solvency of waters from peaty districts is attributed. Experiments to determine the longevity of the typhoid bacillus in various soils have given variable results. Thus, Savage found it to live in highly-polluted, unsterilised mud from a tidal river for two weeks in one experiment, and for five weeks in another. Firth and Horrocks found that in a sewage-polluted soil recovered from beneath a broken drain the organism survived for sixty-five days.

When completely frozen, there is an unexpectedly rapid multiplication of organisms. H. J. Conn thinks there may be two groups of soil bacteria, one flourishing in summer, the other in winter.

Nitrogen Fixation.—A number of organisms absorb or 'fix' nitrogen from the atmosphere, notably *Clostridium Pastorianum*, a sporulating anaërobe, some thermophilic bacteria, and the aërobic ovoid organisms *Azotobacter*. *Botrytis cinerea*, *Aspergillus niger*, *Penicillium glaucum* —in fact most fungi—and a pseudo-yeast, Tulare No. 46*b* (Lipman), also fix nitrogen. The fixation of free atmospheric nitrogen by *Lolium temulentum*, or darnel, is due to the fungus which infests it. This grass has no symbiotic root organisms. Leguminous plants possess on their roots little tubercles containing 'bacteroids.' Bacteroids may occur as rods or branching structures. When inoculated into suitable media, they give rise to the nodule bacteria (*B.*, *Pseudomonas* or *Rhizobium radicicola*) which measure about 5 μ by 1 μ. The latter do not form spores, are actively motile, and are strict aërobes. The organisms enter the plant through the root hairs, where on multiplication they produce a filamentous zoogloea which grows into the root and causes production of nodules. These take up atmospheric nitrogen and convert it into a form assimilable by the plant. Atmospheric nitrogen is absorbed in greater quantity in the legume than in artificial media. The plant, so far from welcoming the organisms, offers resistance to their attack; this resistance is most evident where free potassium nitrate is present, when nodules do not form, because the 'nitrogen hunger' is lessened. The association of the plant and bacteria cannot, therefore, be regarded as true symbiosis.

Success has not always followed the use of pure cultures of the nodule bacteria. Bottomley's preparation of *Azotobacter* is in the form of a powder. It remains virulent for months, and has proved highly successful when intelligently used. Bottomley has shown that by associating together *Azotobacter* and *Pseudomonas* a very much greater quantity of nitrogen is collected from the air, and fixed in a form available as a plant food, than can be fixed by them acting independently. He treated moss litter with the nitrogen-fixing bacteria and other soil organisms, and incubated for three weeks. Ammonia was formed, which combined with the humic acid present, making the whole alkaline and producing a large quantity of soluble humates available for plant growth. The peat was then sterilised by heat, and a pure culture of *Bacillus radicicola* and the *Azotobacter chroococcum* added. After a further period of incubation, the peat contained weight for weight about fifteen to twenty times as much humates as were present in two-year-old rotted farmyard manure. With soil rich in nitrogen, bacterised peat is of little use, but on poor soils experimental croppings after its use have proved highly satisfactory. Professor Bottomley describes substances which he calls auximones, which are comparable to vitamines, in the treated peat.

The small amounts of iron and silicates in humus have been shown to assist *Azotobacter chroococcum*, which explains the favourable action basic slag has on the organism. Bottomley says a good medium for both *Azotobacter* and *Pseudomonas*, or for a mixed culture of the two, may be obtained by adding to distilled water 1 per cent. of dextrin, 0·2 per cent. of dipotassium phosphate, 0·02 per cent. of magnesium sulphate, and 0·4 per cent. of basic slag.

A mild activation of the air by pitchblende has been found to increase the amount of nitrogen fixed by *Azotobacter*. Brilliantly successful results have sometimes followed the addition of soil from a place where the nitrogen bacteria were abundant to an area where development of plants was slow.

Nitrification.—The conversion of nitrogenous substances, as found in dejecta, cadavers, etc., into nitrates (the form in which plants absorb nitrogen), takes place in three stages, each being produced by bacteria. (*a*) Ammonisation:

The first products of the putrefaction of proteins by Proteus organisms are further broken up by *B. mycoides*, *B. subtilis*, *B. mesentericus*, *B. putrificus*, *B. fluorescens liquefaciens*, *B. tumescens*, and other organisms, to produce ammonia. (*b*) Nitrosation: The ammonium salts are converted into nitrites by *Nitrosomonas*, a variety of *Pseudomonas* (short, fat, motile organisms). These organisms differ from ordinary bacteria, but have definite life-cycles, which, however, differ with the locality. They appear to derive their supply of carbon from carbon dioxide alone. (*c*) Nitratation: An apparently different class of organism (*Nitrobacter*) from those concerned in nitrosation converts the nitrites into nitrates.

Denitrification.—A few organisms reduce nitrates with production of free nitrogen—*e.g.*, *B. denitrificans* and *Denitrobacterium thermophilum*, the latter growing at 65° C. Others can only carry the action so far as the formation of nitrites and ammonia—*e.g.*, *B. butyricus*, *B. mycoides*, *B. subtilis*, and *B. tumescens*.

Partial Sterilisation of Soil.—By heating soil to 55° to 60° C., protozoa and nitrifying organisms are destroyed, while denitrifying organisms and those that produce ammonia remain alive, the result being enhanced ammonia production. Effects vary very much, according to the nature of the soil and the temperature employed. Speaking generally, the tendency is for both organic and inorganic matter to become more soluble. At high temperatures, such as 250° C., calcium and magnesium become less soluble. It appears that a toxin is produced which, though inimical to plant growth at first, eventually benefits it. Steam has proved most effective, and Russell and Buddin consider that in vegetation experiments a steamed soil should be included as a standard.

Partial sterilisation may also be effected by the application of antiseptics. Lime, formaldehyde, chloroform, toluene, pyridine, calcium sulphide, carbon disulphide, and cresol have been used for the purpose of destroying protozoa and increasing ammonification.

Examination of Soil.—When the deeper layers are to be examined, care must be taken to prevent contamination with the other portions, particularly the upper layers. Fränkel devised an instrument for taking samples from various depths. A borer contains at its lower end a small

cavity, which can be closed up by turning a handle one way, or opened by turning in the opposite direction. The borer is pushed down to the necessary depth; the handle is then turned; the earth enters the cavity; the handle is again turned, enclosing the sample of earth completely; the borer is then withdrawn. The soil is thoroughly mixed with melted nutrient gelatin, which can be poured into a Petri dish, or, better, made into a roll culture by Esmarch's method. Another method is to wash the soil with sterile water, which is examined, as usual, by the plate method.

The existence of fæcal pollution, and whether it is recent or distant, is regarded by Houston as being shown by the following factors: Total number of aërobic organisms; number of spores present; estimation of *B. coli*, *B. Welchii* and streptococci. Houston found that streptococci disappear rapidly, and regards them as indicating very recent pollution; the spores of *B. Welchii*, on the other hand, persist for longer periods.

Tetanus and malignant œdema bacilli are detected by inoculating glucose formate broth, heating to 80° C. in a water-bath for twenty minutes, and then cultivating anaërobically.

The Bacteriology of Air.

No particular organisms can be regarded as characteristic of air. Those that do occur are derived from dry surfaces, and are carried with the dust by air-currents. A diminution in numbers occurs with an increase in altitude, with an increase of distance from towns into country, and in mid-ocean the air is nearly sterile. The species generally found are spores of moulds, yeasts, bacterial spores, and chromogenic bacteria. Owing to the influence of desiccation, *B. coli* is seldom met with. The presence of tubercle bacilli in dust has been abundantly proved. In sputa found on the ground tubercle bacilli were present in 60 per cent. Examinations of sewer air have given discordant results. Some find half the number of organisms present in external air, and that organisms normal to sewage are comparatively rare. Andrewes found streptococci, generally corresponding with the *S. salivarius* type, while the *S. equinus* type is most abundant in fresh London air. He also found numerous *B. coli*. Horrocks

has shown that in the drainage system of an ordinary private house splashing allows the disengagement of bacteria passing down soil-pipes, which bacteria can be carried along a 4-inch drain, against the flow of sewage, for at least 50 feet by ventilation currents.

Gordon uses the *Staphylococcus epidermidis albus* as an index of pollution of the air with material detached from the skin. He also detects pollution by material brought in from the street on boots by the presence of *B. coli*, spores of *B. Welchii*, certain streptococci, and sometimes *B. mycoides*.

Gordon uses a streptococcus as an indicator of contamination of air with saliva. He found the chief organism present in saliva to be a streptococcus, present to the extent of never less than 10,000,000, and sometimes at least 100,000,000, per cubic centimetre. This streptococcus was of the *brevis* type, growing well anaërobically, and best at 37° C., generally clotting milk with the production of acidity, and producing acid in glucose, lactose, fructose, maltose, and galactose media. It reduced neutral red much in the same manner as the colon bacillus —viz., the cherry-red became changed to a yellowish-green, and was generally non-virulent to mice. Its growth in neutral red broth served as a means for isolating it. The method adopted to examine for contamination with saliva was to use 'broth' plates—*i.e.*, a tube of neutral red broth was poured into a Petri dish, exposed to the air, then poured back into the tube, which was incubated anaërobically at 37° C. The change in the broth, together with a microscopical examination, sufficed to show the presence of the streptococcus.

Streptococci appear to bulk largely in domestic dust. C. A. E. Winslow obtained 22,700 acid-forming streptococci per gramme from an average of nineteen samples of dust taken from New York schoolrooms.

Filtration of Air.—*Dry* cotton-wool, a dry Pasteur-Chamberland filter, a sufficient number of bends in a narrow tube, or, best of all, glass-wool and sugar, prevent the passage of organisms.

Examination of Air.—For qualitative or comparative examinations, Petri dishes containing a solid medium may be exposed.

Hesse's apparatus consists essentially of a glass cylinder

70 cm. by 3·5 cm., covered at one end by two rubber caps, the inner one having a hole in its centre 10 mm. in diameter, and at the other end a rubber cork fits in the cylinder. Through this cork a glass tube 100 mm. in length passes, which is plugged with cotton-wool. The cylinder is sterilised for one hour in the steam steriliser. The rubber stopper is removed, and 50 c.c. of nutrient gelatin in a fluid condition is introduced into the tube and rolled out on the sides, as in the preparation of an Esmarch's tube, leaving a somewhat thicker coating along the under side of the cylinder. The cylinder and its fittings are mounted on a tripod stand, and the glass tube which passes through the rubber stopper is connected by means of a rubber tube with an aspirator, the cotton having first been removed from its outer end. The aspirator most suitable for the purpose is the double wash-bottle arrangement, which is conveniently attached to the stand by means of hooks. The outer rubber cap is then removed and the aspirator started. Air is drawn through the tube by suction, the micro-organisms contained therein falling on the gelatin. The amount of air entering is estimated by the capacity of the flasks forming the aspirator. The rate at which it enters is controlled by the flow of the water, which can be regulated by a pinchcock. Hesse advises that the amount and the rate of flow for rooms and closed spaces should be about 1 to 5 litres, passed at the rate of $\frac{1}{2}$ litre a minute. For open spaces 10 to 20 litres is passed at about four minutes per litre. The tube is then capped and the colonies allowed to develop, after which they can be further examined by subcultures.

Petri's Method.—The air is led through glass tubes packed with fine sand, kept in place by plugs of glass-wool, the organisms being retained by the sand filter, and afterwards the sand is pushed out of the tube with a sterile wire and plated out in gelatin. In this method the sand, being insoluble, is troublesome, interfering with a clear view of the colonies.

Frankland's Method.—Except for the substitution of powdered cane-sugar for the sand, the process is the same as that of Petri.

Sedgwick and Tucker's Method.—A special tube, resembling a narrow cylindrical separating funnel, without a stopcock, is used. Powdered cane-sugar is packed into

the narrow part, and kept in place with glass-wool plugs The volume of air having been aspirated through, the sugar is pushed into the wide portion, a tube of melted gelatin poured in, and after the sugar has dissolved a roll-culture is made in the tube.

Chattaway and Wharton's apparatus measures the air driven through a glass tube by a rubber puff-ball bellows. The air is met by a jet of nutrient medium automatically sucked up by the current of air, as in a scent spray.

The Bacteriology of Milk.

Although the 'fore-milk' (the first portion drawn) generally contains bacteria, which have obtained access to the milk ducts since the previous milking, the milk in the udder of a healthy cow is sterile. After leaving the cow, excremental and other particles from the cowshed, the hide, and the milker's hands, start a contamination which does not cease to be augmented from extraneous sources till the milk is consumed. Especially in poor neighbourhoods, flies are prominent factors in the pollution of milk. Cox, Lewis and Glyn have shown that a housefly, while drowning, may shed from 2,000 to 350,000 bacteria. Flies are garbage feeders, and the bacteria carried in or on their bodies are never desirable, and often pathogenic. It is now proved beyond all doubt that flies can and do infect milk with the organisms producing epidemic diarrhœa of infants, typhoid, cholera, and dysentery.

Milk is an admirable medium for the growth of bacteria, and rapid multiplication takes place. Eyre states that the numbers in London are about 3,000,000 to 5,000,000 in December, January, and February; and 20,000,000 to 30,000,000 in June to September. Milk may be the medium by which the following diseases are conveyed to man: Tuberculosis (pp. 62, 64), typhoid fever (p. 101), sore throat (p. 134), diphtheria (p. 115), scarlatina (p. 195), bacillary dysentery (p. 107), cholera (p. 154), Malta fever (goat's milk, p. 145), foot-and-mouth disease (p. 199), and infantile diarrhœa (p. 196). Milk is, in addition, subject to various diseases peculiar to itself.

Blue Milk.—Blue patches are formed on the surface by *B. cyanogenus*, a small motile multi-flagellate bacillus. The organism does not liquefy gelatin, which, however, is stained bluish-green, finally becoming of a dirty greyish

tinge. The growth on potato is a thick, dirty yellow layer, which afterwards becomes blue; the medium is discoloured. It gives an alkaline reaction in ordinary milk, and does not produce coagulation. It usually yields two pigments—one of the ordinary fluorescent type, and the other of a bluish to greyish colour, which becomes more strongly blue up to azure in milk with an acid reaction. Blue milk may also be caused by other organisms.

Red Milk.—Hæmorrhage from the udder, *B. prodigiosus*, *Sarcina rosea*, or *Saccharomyces ruber*, may give rise to red milk, and the last may cause infantile diarrhœa. Generally *B. lactis erythrogenes* (Hueppe) is to be found—a short bacillus, liquefying gelatin. The colonies are of a yellow colour when first seen, but after liquefaction they become rose-red. A yellowish deposit occurs on agar, which soon changes to yellowish-red. The cultures give rise to an unpleasant, sweet smell. In milk the red coloration is developed best when the medium is slightly alkaline and kept in the dark, and is checked by acidity and light. On standing, the cream rises as a yellowish layer and the casein is precipitated, though the reaction remains alkaline and the clear serum is pink.

Yellow Milk.—The best-known organism causing this disease is *B. synxanthus*, a motile rod, curdling milk by means of a rennet-like ferment, which afterwards redissolves the curd and produces a yellow pigment.

Bitter Milk may be due to the ingestion of certain plants by the cow. Among the several organisms capable of producing bitterness is the bacillus of Bleisch, a flagellated, facultative anaërobe, rapidly liquefying gelatin, and producing a thin, flat, greyish growth on agar and potato. In milk it will, after a week, produce transparent yellow streaks below the cream, the milk itself coagulating, and the coagulum being subsequently, earlier or later, almost completely dissolved. The bitter taste arises after the second week; there is no smell; the reaction is acid. At higher temperatures the milk becomes bitter, and gives the biuret reaction after twenty-four hours, while spores are produced which resist boiling for six hours. Conn's micrococcus of bitter milk coagulates milk, and then produces a slimy solution, with a slightly sour and very bitter taste. Trillat and Sauton found bitter milk to contain aldehydes and ammonia.

Stringy or Ropy Milk.—When poured from a jug, ropy milk takes a rope-like form. If a few drops are placed between finger and thumb, and these separated, the ropy milk draws out in a thread. Ropiness may be due to milk coming from an inflamed udder ('garget' milk), in which case the milk is unfit for food. In other cases, where the bacteria concerned have an extracorporeal origin, the milk, 'though abnormal, is quite wholesome, and does not endanger public health' (Board of Agriculture Leaflet No. 266).

Water used for washing churns sometimes transmits the organisms to the milk, or the cows may stand in ponds containing them. They may be derived from dust, straw, mouldy hay, and butterwort.

The following organisms produce stringiness:

B. lactis pituitosi (Löffler).—A stout, slightly curved rodlet, which does not liquefy gelatin (see below).

B. lactis viscosus ('the viscid milk bacillus'—Adametz) is a very short rod, aërobic, and does not liquefy gelatin. Its effect is apparent after four or five days, and is continued for four weeks, by which time the milk corpuscles have practically disappeared, and the milk is transparent. The casein is not precipitated; no acceleration of the process occurs on a rise of temperature, and there is no special smell.

Streptococcus Hollandicus is used in the manufacture of Edam cheese. It does not liquefy gelatin; it renders milk stringy within twelve to fifteen hours at a temperature of 77° F., the milk becoming sour at the same time.

Norwegian *taettemaelk* is made with a 'stringy' milk bacillus.

Soapy Milk.—*B. lactis saponacei* (Weigmann and Zirn) has been found in straw used as litter. It does not coagulate milk, but makes it slimy and slightly ropy, with a faint soapy taste. It grows best at 10° C.

Slimy Milk is attributable to various organisms. *Micrococcus viscosus* (Schmidt-Mühlheim) is of 1 μ diameter, often occurs in wreathed chains of fifteen or more cells, and produces a slime from the milk-sugar. The process seems to differ from that of slime production in wine, in that it forms no mannite and no carbonic acid. *B. lactis pituitosi* of Löffler gives a specific smell, and renders the milk slimy and slightly acid, especially at the lower part.

Salty Milk may be watery (1027 to 1029 specific gravity). It is stated to occur only in connection with inflammation of the udder. It is detected by taste, its high percentage of ash, and by its low percentage of milk-sugar. According to Klenze, 2·4 per cent. of small deposits of calcium carbonate in the milk glands may give rise to sandy milk.

White Mould.—See *Oïdium lactis*, p. 17.

For practical purposes, it may be said that any milk which goes sour rapidly is a bad milk, although the converse is not necessarily true. According to Schatzmann, if a sample of milk be kept for twelve hours at 40° C., and within that time coagulates, it is to some extent defective, and in nine hours no change whatever should appear to have occurred. The presence of colostrum is a ground for the condemnation of milk; it can usually be detected by the presence of long, elastic, yellowish threads.

Sterilisation.—Fractional sterilisation is too lengthy a process for use commercially, so recourse is had to Pasteurisation (see 'Aids to the Analysis of Food and Drugs')—*i.e.*, the milk is heated to 62° or 68° C. for thirty minutes.* This kills all, or nearly all, the pathogenic bacteria that are likely to be present in milk, but also destroys the lactic acid bacteria. After pasteurising, the spore-forming bacteria which will escape develop, unchecked in the absence of lactic acid bacteria, and may turn the milk rotten, often with no outward sign of its condition. For this reason milk once pasteurised should be consumed within eighteen or twenty-four hours—*i.e.*, before sporulating bacteria have had time to multiply to an objectionable extent. Pasteurisation affords milkmen a convenient method of preserving surplus milk till the following day, but the period during which milk remains in a house is so short that, as a general rule, probably twenty-four hours do not elapse between Pasteurisation and consumption.

Reporting on the electrical treatment of milk as applied by E. W. Hope, Beattie says that there is a reduction in content of bacteria of 99·25 per cent., and colon and tubercle bacilli are destroyed.

* See also p. 60. Sir John McFadyean (Veterinary Congress, 1914) also advocates raising milk to 85° C., and considers there is evidence, already ample in amount, that milk so treated is from a nutritive point of view in no way inferior to uncooked milk.

The Budde process involves the addition of hydrogen peroxide, and the heating of the milk for three hours to 52° to 53° C. Meat extract removes the peculiar taste left by the treatment. In Behring's method 1 ounce of perhydrol is added to 6 gallons of milk, and the milk is then heated to 122° F. Behring believes that milk loses some of its best qualities when exposed to daylight. He advocates green or red milk-bottles.

In the summer months it is a common practice to run the milk as soon as possible through a cooler, which serves to delay bacterial development. One of Houston's 'counsels of perfection' is the cooling to, and maintaining at, a temperature of 10° C. of the milk. The standard of the Academy of Medicine of Toronto requires the milk to have been cooled to 45° F. within half an hour after milking, and kept at a temperature not exceeding this till delivered. They also impose an age limit of twenty-four hours.

Bacteriological Examination of Milk.

Sediment.—This examination is carried out, not only as a qualitative test, but also to obtain an idea of the number of leucocytes present. It may safely be said that no one can differentiate between a pus cell and a leucocyte in a milk sediment, and it is only when the cells occur in excessive numbers, or accompanied by pyogenic organisms, that pus can be certified as present. At the same time, it must be remembered that Revis and others have shown that a very large leucocyte count can be obtained from the milk of healthy cows. Uncertainty exists as to the significance to be attached to the relative numbers of leucocytes and streptococci. Savage (*Journal of Hygiene*, April, 1906) has shown the absence of any relationship between these in the milk of healthy cows; but he also calls attention to the fact that the number of pus cells present in milk from inflamed udders would be much higher than in the milks he examined.

A number of different processes for estimating leucocytes, allowing varying degrees of inaccuracy, are in use, with the consequence that the data have little significance to anyone except the operator. Savage (*ibid.*) dilutes the milk with Toisson's fluid, and, after centrifuging, counts the leucocytes in the Thoma-Zeiss counting

apparatus used in hæmatological work. This method seems to allow a closer approach to accuracy than most others. Houston, in his report to the L.C.C., dilutes the 'filth' obtained by his apparatus, and makes definite-sized cover-glass preparations with 0·01 c.c. of the sediment. The number of cells found in the sediment bears no constant proportion to the total number present in the milk (Breed).

Examination of Sediment.—The strippings, or milk last drawn, is the best for examination purposes (Mettam). The milk is centrifuged for twenty minutes at 1,500 revolutions per minute. At about half-time, the centrifuge is stopped and the cream stirred up in the upper part of the milk. This disentangles a good many organisms, cells, and other matter, that have been lifted up by the fat globules. The centrifuging is completed, and the fat and liquid tipped out. Three slide preparations are made, each with four drops of the sediment, which are spread evenly over three-fourths of the slide. The slides are air-dried, and then treated with a mixture of absolute alcohol and ether for ten minutes. One slide is stained with Löffler's blue, another by Gram's method, and a third by the Ziehl-Neelsen method. The Löffler's blue specimen gives a general idea of the number and kinds of bacteria and cells present. The number of leucocytes in twenty fields should be counted, and if they average more than twenty per field (with a $\frac{1}{12}$ inch), it is pretty certain that pus is present. This is confirmed if in this and the Gram specimens large numbers of streptococci of the *longus* type are found. Confirmatory evidence is obtained if in a fresh specimen (undried) of the sediment mounted in a drop of Gram's iodine solution red blood-cells are seen in addition. The sediment should also be examined fresh with the low powers ($\frac{2}{3}$ and $\frac{1}{6}$ inch) for gross and filth contaminations—*e.g.*, hair, straw, sand, vegetable matter, etc.

The slide stained by the Ziehl-Neelsen method should, after treatment with acid, be treated with alcohol (p. 74) to decolorise any smegma bacilli, which are sometimes found in milk. Unless tubercle bacilli are found before, this slide should be searched for at least half an hour. It must be noted that milk may contain such acid-fast saprophytes as the Timothy-grass bacillus, and,

on the contrary, tubercle bacilli may be missed. Should organisms morphologically resembling the tubercle bacillus be found in a sample from a herd, inspection of the beasts will often reveal an animal showing signs of the disease. The milk from such must be proscribed, pending the result of an animal experiment: Two guinea-pigs are inoculated, one subcutaneously, one intraperitoneally, each with 1 to 2 c.c. of the sediment.

Although probably mistakes do not often occur, the guinea-pig test is not infallible. The experimenter may be misled by the lesions produced by Timothy-grass bacilli, Rabinowitch's bacillus, the 'Mistbacillus' or Johne's bacillus. Conversely, error may occur in the opposite direction, for negative results may be obtained with guinea-pigs inoculated with milk from tuberculous udders. The test usually occupies four or six weeks. In spite of these disadvantages, it is the most reliable method at present known for detecting tubercle bacilli.

Examination for the Diphtheria Bacillus.—A number of serum-tubes are inoculated. If an organism resembling the Klebs-Löffler bacillus be isolated, it must be submitted to the test of inoculation, since bacilli are not infrequently present in milk and milk products which, though resembling the Klebs-Löffler bacillus morphologically and culturally, are non-virulent.

Examination for the Typhoid Bacillus.—The milk can be examined by one of the methods described under 'The Examination of Water.'

Enumeration of Organisms.—This is not generally performed as a routine test, and there are various objections to attempts to use it as an indication of purity. Should, however, a count be desired, plates must be made on the decimal system to show a range of organisms from 20,000 to 20,000,000 organisms per cubic centimetre. Dilutions of from 1 in 1,000 to 1 in 100,000 should be made. Eastes recommends distilled water agar.

Counts made directly on microscopical preparations of milk are higher than those obtained by plating (Brew).

Estimation of B. Coli.—Quantities of 10 and 1 c.c. of milk are inoculated into MacConkey bile-salt lactose peptone medium tubes, and decimal dilutions containing from 0·1 to 0·000001 c.c. of milk are inoculated into tubes of the same medium, using the same sterile 1 c.c. pipette,

and commencing with the lowest dilution (No. 6) and working up to the dilution containing 10 per cent. of milk (No. 1). Tubes showing gas and acid are worked up for typical *B. coli*, as described under 'Water.'

Enumeration of B. Welchii.—Quantities of 100 and 10 c.c. of milk are placed in sterile tubes, and into milk-tubes the following quantities are inoculated: 1 c.c., 0·1 c.c., 0·01 c.c., and 0·001 c.c. The tubes are then treated as described on p. 87.

Enumeration of Streptococci.—The dilutions used in the examination for *B. coli* can be plated out on such solid media as Conradi-Drigalski or bile-salt neutral red lactose agar plates (see remarks on bile-salt media for streptococci, p. 233). Streptococci appear on both as small colonies, which should be subcultured and further examined. Or the dilutions of milk may be inoculated into glucose neutral red broth (first recommended by Savage), and the tubes examined for chains, after incubating at 37° C. for twenty-four to forty-eight hours.

Houston separates the tests into two classes: multiplying factors (*B. coli* and streptococcus tests, and enumeration of organisms), and non-multiplying factors (*B. Welchii* [*Enteritidis sporogenes*] and readings of volume of sediment).

The Toronto Academy of Medicine has fixed the following standard: Milk shall not contain during the months of June, July, August, and September, more than 10,000 bacteria per cubic centimetre, as shown by a forty-eight-hour culture on nutrient agar medium at 37° C.; nor in the remaining months of the year more than 5,000 bacteria per cubic centimetre, as demonstrated by the same test.

CHAPTER XXI

THE BACTERIOLOGY OF WATER

CERTAIN organisms are so frequently found in water that they may be regarded as normal inhabitants thereof, others are recognised as soil bacteria that have been washed in, while the third group (bacterial indicators of pollution) comprises organisms of intestinal origin. The bacterial alvine flora of animals and birds resembles that

of man, with the consequence that it is often impossible to identify the source of pollution by bacteriological results.

Typhoid, cholera, paratyphoid, dysentery, and perhaps anthrax, are the principal water-borne diseases. Poliomyelitis, sore throat, conjunctivitis, suppurative otitis media, and frontal sinus suppuration, are all supposed sometimes to follow visits to swimming-baths used by infected persons.

Several factors contribute to the increase or decrease of the number of bacteria in water. Sunlight (p. 9) plays an unimportant part in reducing the number, unless the depth of water is very shallow and exposure is prolonged. Cold inhibits growth of bacteria, and, *ceteris paribus*, a higher bacterial content should be found in warm weather. Other influences may, however, cause the winter content of bacteria to be greater than that of summer. When food material is ample in quantity more organisms are met with. Most bacteria tend to settle to the bottom of a bulk of water, and this sedimentation constitutes an important factor in the self-purification of waters. Storage is an important process in the purification of water on a large scale. By storage time is given for any typhoid or cholera bacilli to die out, owing to lack of nourishment, to a temperature unfavourable to development (in temperate climates), and to antagonism of normal water bacteria.

In the M.W.B. reservoirs, Thames and Lea waters can be stored for fifty-two days. The reduction in the number of organisms is sometimes over 99 per cent. It has been suggested that, owing to bacteria clumping during storage, and each clump producing a single colony, and therefore being counted as one organism, the diminution is more apparent than real. While this may be the case, clumps of bacteria settle quicker than do individuals, and the clumping may accelerate sedimentation (see p. 99).

Houston found that when typhoid bacilli or cholera vibrios were added to raw Thames, Lea, or New River waters, a reduction of 99·9 per cent. of these organisms in each case was attained in a week. Cholera vibrios could not be found in three weeks, but eight weeks' storage was found to be necessary for the disappearance of *B. typhosus* (*cf.* p. 99). This bacterial purification is

known as the ' safety change.' Perhaps the tendency for these two organisms to rise to the top of a bulk of water, where the influence of light is possible, allows sunlight to materially contribute to their ultimate disappearance.

Houston considers that the ' safety ' of an adequately stored water may possibly come to be ' accepted so fully as even to afford justification for filtration through mechanical filters at a specially rapid rate, with the result of thus compensating to a large extent for the initial cost of the storage reservoir.' Houston's final conclusion is that raw river water should be stored antecedent to filtration, preferably for thirty days. Prescott and Winslow (1913) conclude ' that any water which has been stored for four weeks is practically safe.'

A pure water when freshly drawn, even if kept in a sterile flask free from aerial contamination, will (after a slight diminution in bacterial content during the first three to six hours) exhibit a remarkable increase in the content of bacteria. Less pure waters, such as those of ordinary rivers, which contain initially a large number of bacteria, exhibit, when similarly treated, a much less conspicuous increase in their bacterial population. Frankland points out, however, that whilst in sewage the number of organisms only gradually diminishes, in pure waters ' after the rapid increase in numbers follows a correspondingly rapid decline, so that the numbers again fall below those found in impure surface waters.' Multiplication after sampling appears to be less in a large sample than in a small one, and less when no air is admitted than with free aeration. The composition of the bottle and the temperature at which it is kept also influence the rate of multiplication.

Collection of Water Samples.—Samples should be collected in sterile glass-stoppered bottles. In sampling a lake or stream, the bottle should be immersed with the neck a foot below the surface and away from the edge before the stopper is removed. A tap should be allowed to run for five or ten minutes before a sample is taken, as bacteria may multiply while water stands in the pipes. When sampling a public supply, a tap directly off the main or on a hydrant should be used, as passage through a cistern introduces a factor of contamination unfair to the water. Samples should only be taken from pumps

after pumping has been carried on for some time. A sample from definite depths can be collected by small vacuum bulbs, which are let down to the necessary depths by means of a weighted wire or string; the drawn-out point of the bulb is then broken by a suitable mechanical arrangement (Sclavo's 'smash bottles'). If more than three hours must elapse between sampling and commencement of examination, the bottle must be kept or packed in ice.

Bacteriological Examination of Water.

Although examination for a specific pathogenic organism is sometimes required, bacteriological examination of water is generally directed at the demonstration of absence or presence of sewage contamination. The important water-borne diseases being usually of intestinal origin, absence of sewage contamination shows fitness for drinking purposes, unless of course chemical examination has shown metallic or other poison or excessive mineral salts to be present. Of course, no examination is a criterion of permanent fitness for use where intermittent or sporadic pollution is possible.

A bacteriological examination may fail to indicate a dangerous water where urine, but not fæcal, contamination occurs. In such a water there may be a high count, but the absence of intestinal organisms will probably produce a favourable bacteriological opinion. This form of contamination is not so rare as is usually supposed, and epidemics have followed when typhoid bacilluria has contributed to it (see p. 99). Colon bacilli and streptococci will also be absent from water receiving drainage from a disused cesspool.

Bacteriological examination will also fail when water has been heated, treated with antiseptics, or filtered, for the purpose of misleading the bacteriologist.

A scheme drawn up by the committee appointed by the Royal Institute of Public Health is very generally followed in this country, according to which the irreducible minimum of procedures should be—

(*a*) Enumeration of bacteria capable of growth at room-temperature (18° to 22° C.). (*b*) Identification and enumeration of *B. coli*, if present.

The majority of the committee recommend, in addition:

(*c*) Enumeration of bacteria capable of growth at blood-heat. (*d*) Enumeration of streptococci, if present.

B. Welchii is not often included among the bacteria estimated, but cases occur where an estimation is desirable.

Enumeration of 'Cool' Organisms.—All media used in counts should have a reaction of + 10 (Eyre's scale). Nutrient gelatin is most often used for the 'cool' organisms, as it gives a relatively larger number of organisms with a polluted water than does distilled water gelatin. Conversely with an unpolluted water, a larger count is usually obtained with distilled water gelatin than with nutrient gelatin. So, although nutrient gelatin should be used when only one gelatin medium is employed, the use of both gives comparative figures of value.

Three tubes of nutrient gelatin are melted in a water-bath at blood-heat or else in a blood-heat incubator. Three sterile Petri dishes are inoculated with 0·5, 0·3, and 0·2 c.c. of the water respectively and labelled. After wiping the adherent water from the outside and flaming the mouth of the tube, the contents of a gelatin tube is poured into each inoculated Petri dish, and the water and gelatin mixed by tipping the dish back and forth a few times. The plates are allowed to solidify on a flat surface, and then placed in the cool incubator. Counting should be done at the end of seventy-two hours, but plates should also be inspected daily, as the count may have to be made earlier should too many liquefying organisms be present. Counting is done with the naked eye, preferably in daylight, any doubtful colony being determined with the aid of a lens or low-power objective.

In a pure water it will generally be found that less than 10 per cent. of the organisms liquefy gelatin. With a polluted water the ratio of the number of organisms developing at room-temperature to those developing at blood-heat approaches 10 to 1, and frequently becomes 10 to 2, 10 to 3, or even less. But, as Horrocks has pointed out, the number of *B. fluorescens liquefaciens* and *non-liquefaciens* may be abundant, and as they grow well at blood-heat, not only may the ratio of liquefying to non-liquefying organisms be misleading, but also the ratio of 'blood-heat' to 'cool' organisms be rendered of no value for an opinion.

In the purest upland streams and lakes the number of bacteria in 1 c.c. is frequently under 100, while in town sewage there are many millions in the same volume. In ordinary rivers the number is generally between 1,000 and 100,000 per c.c. In water from deep-seated springs the presence of more than 100 organisms per c.c. is conclusive evidence of some contamination with surface water.

In waters of poorer quality the numbers may approach 500 per c.c. Anything over this casts suspicion on the water, and 1,000 per c.c. or more should probably condemn the sample, always supposing, of course, that multiplication *in vitro* can be excluded by the proper storage of the sample bottle in ice. In Victoria aerated waters may not contain more than 40 bacteria per c.c.

Before taking a portion of the sample for any purpose the water should be thoroughly shaken, the American committee interpolating the procedure, 'Shake at least twenty-five times the bottle which contains the sample.' They also stipulate that the 'cool' incubator should be well ventilated, and that its atmosphere should be practically saturated with moisture. To avoid fictitious accuracy they stipulate that numerical results should be returned to the nearest 5, 10, 25, etc., number of organisms depending on the number found. Counting a fractional part of the organisms is sometimes done when plates are crowded, by means of a Pakes's disc, but it is far preferable to count all the organisms. As a thousand organisms can be counted on a 10-centimetre dish, there is generally little need to dilute, and if the organisms exceed this on a plate made from 0·2 c.c., they can be truly returned as 'uncountable.'

Crowded plates are to be avoided, as, quite apart from the labour of counting, the results tend to be low: some colonies may be hidden under others, two colonies may coalesce and only count as one, and the metabolic products excreted by quick-growing species may diffuse into the medium and inhibit growth of neighbouring colonies. Therefore it is desirable that 200 colonies should be the maximum allowed on a dish, and when dealing with a water known to contain a large number of organisms, plates should also be made from dilutions of the sample. It is very desirable that duplicate sets of plates should be made for all counts.

Enumeration of 'Blood-Heat' Organisms.—Two sterile Petri dishes are inoculated with 1 and 0·1 c.c. of the sample respectively. Two tubes of nutrient agar are melted in a bath of boiling water and allowed to cool to 45° C., when the tubes are wiped on the outside, the mouths flamed, and the contents poured into the Petri dishes. The agar and water are mixed by tilting, and placed on a flat surface to cool. This needs to be done with expedition (p. 42). The American committee recommend the use of Petri dishes with 'porous earthenware covers, in order to avoid the spreading of colonies by the water of condensation.' Otherwise the agar plates should be inverted in the incubator. Counting should be done at the end of forty to forty-eight hours.

The actual number of organisms capable of development at blood-heat is subordinate in importance to the ratio of organisms developing on the agar plates to those developing on the gelatin plates. In a pure water this is generally considerably less than 1 to 10—*i.e.*, 1 to 20, 30, or 40—while in an impure water the ratio becomes 1 to 8, 5, 3, or more.

Estimation of B. Coli.—Quantities of the sample amounting in all to 50 c.c. for a shallow well or surface water, or to 100 c.c. for a deep-well water, are added to tubes of a medium containing some substance that is fermented by the colon bacillus, and some other substance that, while inhibiting the growth of most water organisms, allows the growth of the colon bacillus. Savage prefers neutral red glucose broth, but MacConkey's bile-salt broth is generally used, and the carbohydrate incorporated is usually lactose. The colon bacillus ferments this sugar with production of acid and gas, a fermentation reaction not given by a number of organisms that produce acid and gas from glucose. The composition of the broth as described on p. 237 is of 'single strength.' A quantity of medium containing double the amounts of the constituents is also prepared—'double strength.'

If the water from a shallow well is to be examined, quantities of 0·1 and 1 c.c. of the sample are inoculated respectively into two tubes containing 10 c.c. of single strength medium. Tubes containing respectively 5 c.c., 10 c.c., 15 c.c., and 20 c.c. of double strength broth are inoculated with 5 c.c., 10 c.c., 15 c.c., and 20 c.c.

respectively of the sample. When a water to which a more stringent standard for *B. coli* can be applied is to be examined, quantities of 10 c.c., 20 c.c., and 20 c.c. are, in addition, added to double-strength tubes containing similar quantities of the broth. This will give a total quantity of water examined of over 100 c.c. The tubes are then incubated at 37° C.—or, better, at 42° C.—for twenty-four to forty-eight hours. The production of acid and gas is only *presumptive* evidence of the presence of *B. coli*. Other organisms are frequently responsible for the change, hence the necessity for the isolation and identification of the organism. Contrariwise, the colon bacilli may not give the reaction for seventy-two hours, or they may be inhibited or killed by the bile-salt, but in these cases the bacilli may be regarded as attenuated forms and their non-detection as of no serious consequence. Negative results are very occasionally due to death or inhibition of colon bacilli through overgrowth of streptococci. This only seems to occur with grossly polluted waters, especially when large quantities of water (such as 100 c.c.) are tested (Prescott and Winslow).

The tubes which received least water, and which show acid and gas, are used for making the secondary cultures for which neutral red bile-salt agar or Conradi-Drigalski agar are convenient. The use of Petri dishes for these secondary cultures is not necessary. A loopful of liquid from a primary tube showing acid and gas is added to a test-tube containing 10 c.c. of sterile water. This is agitated, and a loopful transferred to another test-tube of sterile water. The latter is agitated, and a loopful added to a third tube of sterile water. A loopful of the first dilution is smeared over the surface of a sloped tube of medium, and similar quantities of the other two dilutions are smeared over the surfaces of two other sloped tubes. One or other of these secondary cultures is pretty certain to give discrete colonies. Characteristic colonies are subcultured into tubes of nutrient broth, and when growth is sufficient the broth cultures are examined for the presence of motile organisms, and preparations are examined by Gram's method. The attributes of a typical colon bacillus are given on p. 90. From the broth culture inoculations into the various media necessary to identify the organism are made. The estimation of *B. coli* is

unanimously regarded as the most important datum obtained in the ordinary bacteriological examination of water. If the colon bacillus is found, it is usual to report it as absent in such a quantity, but present in so many cubic centimetres. Thus, if typical *B. coli* be found in the tube to which 10 c.c. of the water had been added, but that receiving 5 c.c. remained unchanged, the organism is present in 10 c.c., but absent in 5 c.c.

Savage came to the following conclusions (1902), the justification of which is very generally conceded: Waters which show no *B. coli* in 50 c.c. are of a high degree of purity, and therefore the proved absence of this organism in this amount, and still better in larger quantities, is of great value. *B. coli* should be absent from at least 50 c.c. of spring water, possibly from greater amounts. In upland surface waters the presence of *B. coli* in 40, 10, or even 2 or 1 c.c., means contamination, but not necessarily a contamination which it is essential to prevent. It may be from contamination with the excreta of animals grazing on the gathering areas, and is by no means necessarily from sewage or other material containing specific organisms of infection. If *B. coli* are present in numbers greater than, say, 500 per litre (or even in that amount), such a water is suspicious, as it is rare to get so many *B. coli* in a water from the kind of animal contamination indicated, and further investigation is desirable. In filtered samples the number of *B. coli* is as a rule considerably reduced. In surface wells *B. coli* in large numbers indicate surface or other contamination, generally very undesirable, if not actually dangerous.

Deep-well or spring water should not usually contain *B. coli* in 100 c.c.

As regards atypical *B. coli*, we cannot do better than again quote Savage: 'The nearer these . . . approach typical *B. coli* in their characters, the more nearly are our numerical standards for that organism applicable to them, while if they lack essential characters, a proportionately greater number must be present to justify an adverse opinion.'

Enumeration of Streptococci.—Quantities similar to those used in the estimation of *B. coli* are inoculated into glucose broth or glucose neutral red broth, and incubated at 37° C. for forty-eight hours, when hanging-drop or

Gram-stained preparations are made from the bottom liquid. Or the actual tubes used for the primary examination for *B. coli* may be examined.

Not all streptococci will grow in the presence of bile salt, but as the group that is important in the sanitary analysis of water—*i.e.*, those from human excrement—does, omission to find others is of little consequence.

For the isolation of streptococci, Conradi-Drigalski agar is convenient. Houston showed that fæcal streptococci from human sources produce acid from lactose and saccharose media, and generally from salicin too. They produce acid and clot in milk, produce a uniform turbidity in broth, and reduce neutral red—that is, they correspond to the *S. fæcalis* of Andrewes and Horder (p. 134). Houston found no reliable distinction between the streptococci of man and animals. With the horse, sheep, rabbit, and cow, excrement is deficient in streptococci, and especially in lactose-positive streptococci as compared with human excrement.

It is important to note that the human excrement group of streptococci is of a *brevis* type. If, in the examination of a Gram-stained slide, Gram-positive diplococci are noticed, the search should be kept up, since perhaps these diplococci are streptococci that have not grown out. In most of such cases definite chains will be found sooner or later.

In contradistinction to *B. coli*, streptococci do not multiply in water after sampling (non-multiplying factor). Previously regarded as indicators of recent pollution, owing to an idea that they soon died out in water, they are now regarded by Horrocks and most other workers as living as long as, if not longer than, *B. coli*. Their number should not exceed that permissible for *B. coli*.

Enumeration of B. Welchii.—Quantities amounting to 250 c.c. for a surface water, and up to a litre for a deep-well water, are examined as described on p. 87. This organism is a ' non-multiplying factor.' Savage considers that it should be absent from 100 c.c. of a surface water and from a litre of a deep-well water. The estimation is not usually included in routine examinations, owing to the uncertainty as to its interpretation. Thresh has, however, suggested a number of standards (see ' Applied Bacteriology ').

Detection of the Typhoid Bacillus.—The incubation period of typhoid fever is generally ten to fourteen days, a period often long enough for the disappearance of the organism from the water before an examination is suggested, leaving out of the question the period elapsing before the water is suspected. A water containing typhoid will almost certainly contain very much larger numbers of the colon bacillus and other organisms which will flourish on the media used for isolation of the typhoid bacillus, crowd the plates, and perhaps outgrow the organism sought. The last reason is less likely where typhoid urine alone is responsible (see pp. 99 and 227), and this class of cases happens to be that where typhoid bacilli have been isolated and successfully identified. Apart from the urine contamination cases, there has been constant failure to find the typhoid bacillus in incriminated water, and considering all things this is no matter for surprise. A negative result is worthless, and great danger to the community may result from failure to realise this.

The amount of actual typhoid pollution is almost invariably very small, and direct plating of a suspected water is waste of time. One of the following methods of concentration is therefore adopted:

(*a*) *Filtration through a Porcelain Filter.*—A large number of the organisms get entangled in the filter candle, and the process is seldom used.

(*b*) *Willson's Precipitation Method.*—A 10 per cent. solution of alum in sterile distilled water is added to the water till it contains 0·5 gramme of the salt to the litre. The precipitate is allowed to settle, or, after the precipitate of aluminium hydrate has formed, the vessel is well shaken to distribute its contents evenly, and centrifuged for fifteen minutes. The clear supernatant fluid is then siphoned or poured carefully off from the precipitate, and the mass of precipitate in the conical extremity of the tube stirred up with the little fluid remaining. The suspension is then plated out. If the water is very soft it is advisable to add a little lime-water, or the precipitate will not form well. The alum is said to have no bactericidal effect, but if too much alum be allowed on the surface of a plate growth will be retarded. This method is the most satisfactory.

(*c*) *Serum Agglutination* (*Schepilewsky*).—Ten to twenty

c.c. of the water are added to 50 c.c. of nutrient broth, to which, after three or four days' incubation at 37° C., an addition of the typhoid serum is made, and after standing for some hours and centrifuging, the deposit is plated out.

(*d*) *Hoffmann and Ficker's Enrichment Method.*—The water itself is converted into a nutrient medium by the addition of 1 per cent. of nutrose, 0·5 per cent. caffeine, and 0·001 per cent. of krystal violet. The mixture is incubated at 37° C. for twelve to thirteen hours, by which time the typhoid bacilli should have multiplied to such an extent as to permit of direct isolation by plating, the *B. coli* being inhibited. It is very doubtful whether this medium is really satisfactory. The action of caffeine on *B. coli* is uncertain, while krystal violet merely inhibits water organisms.

(*e*) *Process of Cambier.*—A special alkaline peptone medium is placed in a glass jar. In this is immersed a Pasteur-Chamberland filter candle half filled with the same solution, to which is added a little of the fluid to be examined, and the whole is incubated at 37° C. Sooner or later growth appears in the fluid outside the candle, and Cambier states that if typhoid bacilli be present they will make their appearance before *B. coli*. Most of those who have tried this process find that organisms will grow through, but they are usually not *B. typhosus*.

(*f*) Wilson adds 10 c.c. of nutrient broth to every litre of the water, and evaporates at reduced pressure at 42° C.

Isolation of the Typhoid Bacillus.—The concentrated deposit obtained is plated out on a medium chosen for the more or less characteristic growth of typhoid bacilli thereon, and for its ability to inhibit the growth of most other organisms. Conradi-Drigalski agar, litmus lactose bile-salt agar, Fawcus's medium, China green agar, or one or other of a host of media all devised for this purpose, may be employed. Of one thing a bacteriologist may be certain: he will always get colonies portraying the characters of a typhoid colony on that medium. These colonies must all be subcultured into broth and their attributes worked up (see pp. 97 and 108). If plenty of typhoid serum is available, it is advisable to try agglutinating reactions on each subculture as soon as possible. Pfeiffer's test should be done to clinch a diagnosis. It

must be borne in mind that typhoid fever, as diagnosed clinically, is not always due to a typical Eberth-Gaffky bacillus, and should an organism be isolated differing in one or two respects from the recognised characters of this bacillus, the agglutination test should be performed with the serum of a case supposedly infected through the water. With an atypical typhoid bacillus a negative Pfeiffer reaction with an animal receiving this bacillus and the serum from an animal immunised against a typical typhoid bacillus is of doubtful significance, as the reaction is such a markedly specific one.

The following organisms resemble the typhoid bacillus morphologically and in cultural characters:

Bacillus aquatilis sulcatus is distinguished from the typhoid bacillus (1) by developing at 5° C.; (2) by growing feebly at 37° C.; (3) by not agglutinating with typhoid serum; and (4) by the colonies after a time acquiring a yellowish colour. Several varieties have been described.

Bacillus fæcalis alkaligenes does not agglutinate with typhoid serum, and produces alkali in milk. Fuerth describes a disease simulating typhoid produced by an organism of this type. Savage mentions a single sporadic case of gastro-enteritis that was shown by Ridder to be probably caused by this organism.

B. coli anaerogenes is dealt with elsewhere (pp. 93 and 108).

Detection of the Cholera Vibrio.—See p. 151.

Media used in the Examination of Water.—The media used for enumeration purposes should preferably be not more than three weeks old, as changes occur in the reaction with age. Such media are to have a reaction of + 10.

Nutrient Agar and *Nutrient Gelatin.*—See p. 36.

Distilled-Water Gelatin.—Ten per cent. gelatin in distilled water.

Distilled-Water Agar.—Powdered agar 1½ per cent., dissolved in distilled water.

Peptone-Water and Carbohydrate Peptone-Waters.—See p. 37.

Litmus Milk.—See p. 38.

Neutral Red Broth.—A ½ per cent. solution of neutral red in water is added in the proportion of 1 c.c. to every 100 c.c. of a broth containing 0·5 per cent. of glucose.

MacConkey and Hill's Bile-Salt Broth.—Sodium taurocholate 0·5 gramme, glucose or lactose 0·5 gramme, peptone 2·0 grammes,* are dissolved in 100 c.c. of water by heating; the mixture is filtered, and after filtration sufficient neutral litmus solution is added to give a distinct colour. The medium is then distributed into Durham's fermentation tubes, and sterilised for twenty minutes on three successive days. This is of 'single strength.'

Neutral Red Bile-Salt Lactose Agar (MacConkey).—Sodium taurocholate 0·5 per cent., peptone 2 per cent.,* lactose 1 per cent., agar 2 per cent. To be made with tap-water, and 0·5 c.c. of a 1 per cent. solution of neutral red added.

Organisms, such as *B. coli*, which ferment lactose generally, but not invariably, give bright red colonies. *B. typhosus*, which does not ferment this sugar, produces white colonies, unless the peptone contains glucose.

Conradi-Drigalski Agar.—Add peptone 10 grammes, nutrose 10 grammes, sodium chloride 5 grammes, to 1 litre of acid beef broth. Steam for one hour, and add 25 grammes of powdered agar. Steam for three hours, bring to a reaction of + 10, and filter through *papier Chardin* (*A*).

Boil for a few minutes 100 c.c. of Kubel-Tiemann litmus solution, add 15 grammes of pure powdered lactose, and boil again for a few minutes (*B*).

Add *B* to *A*, and to this mixture add 2 c.c. of a hot 10 per cent. solution of anhydrous sodium carbonate and 10 c.c. of a 0·1 per cent. solution of krystal violet. The medium is then tubed, 10 c.c. being placed in each test-tube, and sterilised. On this medium in forty-eight hours *B. coli* forms large red colonies, typhoid small blue 'dewdrop' colonies, and streptococci small delicate red colonies.

This medium and the one preceding are employed as *surface* plates. Tubes are melted in a water-bath, and their contents poured out into sterile Petri dishes. When set, the plates are placed in the blood-heat incubator for two hours, with the lids slightly tilted at one edge, so that the surface of the medium may dry somewhat. Otherwise the moisture present would probably cause the

* The peptone used must be free from glucose.

colonies to run together. The matter to be plated is sufficiently diluted, and one or more drops are run on to the surface, and spread by means of a sterile glass rod bent at a right angle.

China Green Agar (*Werbitzki*).—An ordinary 3 per cent. agar neutralised to + 13 on Eyre's scale, with 1·4 to 1·5 c.c. of a 0·2 per cent. solution of China green added to each 100 c.c. contained in a flask. McWeeney says that China green agar suppresses about 75 per cent. of the coli colonies, whilst the survivors are much inhibited in their growth, remaining small, opaque, and point-like. The typhoid colonies, on the other hand, develop most luxuriantly, and are decidedly more numerous than on any of the other media. Their appearance is of a delicate transparent green, deeper in the centre, with a thin, filmy, peripheral layer that spreads out like a veil over the substratum, and presents under a low power a typically sulcate appearance. The motility of the typhoid bacilli is much diminished, and they present a filamentous aspect. Subcultures in broth, however, are typical by the next day, and can be subjected to the usual agglutination and other tests.

Filtration of Water.

Domestic Filters.—The primary object of a filter is to remove suspended matter, including micro-organisms. The filtering media include carbon (animal and vegetable charcoal, silicated carbon, and manganous carbon), felt, sponge, carferal (charcoal, clay, and iron), spongy iron (Bishof's filter), magnetic iron, cellulose, earthenware, and natural stone. Animal charcoal oxidises and absorbs part of the organic matter, removes colour due to organic impurities, and is credited with the power of removing lead. Vegetable charcoal is less efficient. Iron may reduce nitrates to ammonia. With the exception of earthenware types, these filters only remove part of the bacteria, and, unless the filtering medium be often renewed, may actually be sources of danger. If once polluted with typhoid or cholera, they may subsequently convey the bacilli to unpolluted water for long periods after contamination. The initial efficiency of these filters is due to the bacteria being temporarily arrested in the filter media, where they multiply and are gradually

washed through, the bacterial content of the water increasing until sometimes it is greater than that of the unfiltered water. Sponge filters form an excellent nidus for bacterial growth, and are particularly reprehensible. Bacterial filters of the earthenware type are by far the most satisfactory. These are made in tubes or ' candles,' to offer as large a surface as possible to the water.

The Pasteur-Chamberland candle is a composition of unglazed porcelain, the Berkefeld of a diatomaceous earth (kieselguhr); the Slack and Brownlow, and the Doulton filter, are also composed of porcelain. None continues to give sterile water indefinitely, and should be submitted to a weekly scrubbing with a nailbrush, and subsequent boiling in water containing sodium carbonate. Kieselguhr filters vary considerably in the length of time for which they will give sterile water, some allowing direct contamination, while others are satisfactory. The Berkefeld filter has a small portion of its surface removed every time it is scrubbed, and thus may in time become faulty.

The inevitable appearance of organisms in the filtrate, in the case of all filters after shorter or longer periods of effectiveness, is, according to Craw, partly dependent on a mechanical acceleration, caused by the water current sweeping the micro-organisms through the filter mass. This indirect contamination appears to depend partly on the grain of the filter. The most efficient makes of filters examined by Craw — the Pasteur - Chamberland and Doulton—contain very small-sized pores; the Berkefeld pores are larger, and the Slack and Brownlow larger still. The characters of the organisms (motility, size, etc.) and the chemical composition of the water may also be factors in determining the period of sterility. When water is filtered under pressure an extra strain is put on the filter. The soundness of the Pasteur-Chamberland filter can be ascertained by compressing air in the candle at a pressure of one-half to one atmosphere, when, if held beneath water, no air will escape from a sound tube. A stream of bubbles will issue from any spot capable of passing bacteria.

Recognising their limitations, bacterial filters, especially the Pasteur - Chamberland, offer a perfect protection against infection from drinking-water. The slow rate of filtration is not surprising considering the size of bacteria,

and by using a battery of filters the supply can be increased at will.

Sand Filtration.—Filter-beds at waterworks are constructed of a layer of large stones, with unjointed pipes placed at intervals at the bottom; smaller stones are placed above these, then gravel and rough sand, and lastly a layer of sand from 2 to 4 feet deep. The fineness and contour of the grains of sand, the depth of the filter, and the rate of filtration, all affect the working of the filter in the removal of organisms. The fineness of the sand is *per se* no criterion of filtering power, a comparatively coarse sharp-angled sand acting better than a finer article with rounded edges. Sea sand is for this reason unsuitable for filtration. Koch's coefficients for safe working are filtration through a sand layer not less than 30 centimetres thick, at a rate not exceeding 100 millimetres per hour, and giving a filtrate containing not more than 100 bacteria per c.c. growing on gelatin. These coefficients, however, take no account of the class of sand used or character of water filtered, and they are no longer regarded as trustworthy. When freshly constructed, organisms are washed through a filter-bed with great rapidity, but after a certain quantity of water has passed through or the water has been allowed to stand upon it for a certain time, a slimy coating of detritus, bacteria, and other lowly organisms, is formed on the surface. If water is slowly passed through the filter when sufficient of this coating has formed, the majority of the bacteria will be retained by this surface, either by sticking to it or by being strained off. The increasing thickness of this coating will reduce the velocity with which the water passes, and at the same time some of the bacteria will tend to grow downwards into the lower strata of the filter, and, if the process were continued long enough, would be washed through into the filtrate, and ultimately become more numerous there than in the unfiltered water.

This layer also serves as a culture ground for oxidation bacteria, which to a large extent tend to prevent the multiplication of the other bacteria, and consequently their growth through the filter. In summer, when the temperature is more favourable to the growth of the organisms, the purification is more complete than in winter.

The indication for scraping usually adopted is that the

filter-bed no longer passes the required quantity of water under the maximum permissible head. The sufficiency of this practice has not been clearly shown. No general rule can be given for the depth to which the top layer must be removed, as it varies with the nature of the water and sand, temperature, etc. The epochs of scraping and other interference, as by storm water, with the filter-beds, have been frequently observed to synchronise with groups of typhoid cases in the districts served with the water in question. A properly working filter should remove 98 or 99 per cent. of the organisms, and a filtered water should not contain *B. coli* in 100 c.c.

The sand filters of the Metropolitan Water Board vary in depth from 2 to 4¼ feet, and the rate of filtration varies from 0·89 to 1·94 gallons per square foot per hour. Each acre of filtering surface requires to be cleaned on the average seven times during the year. Filtered Thames and Lea waters contain two to three colon bacilli per litre (Houston).

The Sterilisation of Water.

The use of bacterial filters is dealt with on p. 239. Water may be efficiently sterilised by heat. In apparatus for this purpose, the hot sterile water passes through tubes or compartments surrounded by the incoming water, with the result that the sterilised water is cooled and at the same time the unsterilised water is heated to such a temperature that comparatively less fuel is required to bring it to the necessary temperature. Water sterilised by heat loses part of its natural salts and part of its dissolved gases, and may be mawkish in flavour. For such an apparatus to be safe it must be fitted with an automatic valve which does not allow water to leave the apparatus until it has been raised to the required temperature. Such an apparatus would be safe, but it cannot be cheap in first cost, and its weight and the cost of fuel are against its popularity except on a small scale.

The distillation of water is sometimes practised where water contains too great a quantity of salts to be drinkable. The distillate is, of course, sterile if properly collected, but, like all boiled water, it must be aerated to make it agreeable to the palate.

Bisulphate of sodium (15 grains per pint) is said to

sterilise water in thirty minutes, but the use of an acid compound is to be deprecated. In the British Army tabloids of sodium bisulphate, flavoured with lemon and saccharin, are supplied for the use of cavalry. In practice it is found that men soon get tired of the taste of water treated in this way, and, though the tabloids may be issued, they are not regularly used.

Potassium permanganate is effective, but it is best followed by treatment with alum. Copper sulphate in a very weak solution kills algæ (p. 254), and its use has been suggested for the routine disinfection of water.

Iodine has been employed and is quite effective, but the water must be subsequently treated with sufficient hyposulphite of soda to neutralise the iodine.

Of late years sterilisation of water by chlorine has come into use, and its employment is rapidly increasing. Its use was suggested by Major Nessfield in about the year 1897, who showed that colon and typhoid bacilli could be killed by the addition of chlorine to water in high dilutions. Later it was found that the addition of small quantities of a dilute solution of bleaching powder was equally effective. The less a water contains of organic matter and chemical compounds that combine with bleaching powder, the less is needed for sterilisation.

The essential point is to add as much bleaching powder as is required to kill the disease-producing organism (which is assumed to have occurred if no living coli-group organisms can be recovered after treatment), but to avoid excess, because even a very small excess of bleaching powder gives a most unpleasant taste to water. This taste is brought out more strongly when water is made into tea. Some individuals are very sensitive to small quantities of bleaching powder. Moor and Hewlett, in a report to the L.G.B. (Report of the Medical Officer to the Local Government Board, 1909-10), examined the processes available for the purification of chalk waters, and reported in favour of sterilisation by bleaching powder. In those investigations it was shown that 0·25 part of chlorine (equivalent to approximately 0·75 part of good bleaching powder) per million killed colon bacilli in chalk water in thirty minutes. The amount of chlorine necessary varies. Sims Woodhead found the amount of chlorine necessary to kill the whole of the

non-sporulating bacilli in Cambridge water is usually 1 part per 7,000,000 parts of water. Less pure waters may require 0·7 to 2 parts of available chlorine per million.

The army directs that 2 grammes of bleaching powder shall be added to the contents of water-carts which hold 100 to 110 gallons, at the discretion of the medical officer.

Many water-supplies in America and some in the United Kingdom are now sterilised by bleaching powder. In order to remove any traces of the reagent after treatment, the water may be run through iron turnings or charcoal, or an addition of an appropriate quantity of sodium hyposulphite may be made.

One of us (C. G. Moor) has applied the process of sterilisation of drinking-water on a large scale for camps and hospitals at a base in France, with satisfactory results.

At present it appears that sterilisation by minute quantities of bleaching powder, or by chlorine produced electrically, is the coming method, and its cheapness and simplicity make it applicable everywhere. Before it is applied, waters should be strained or filtered to take out all visible particles, otherwise the sterilising solution may fail to kill bacteria enclosed in such particles. For army use a valuable piece of apparatus has been devised by Colonel Horrocks, in which water is clarified and sterilised by bleaching powder at one and the same time.

Water may be sterilised by allowing it to trickle down a tower through which ozonised air is caused to pass. This is a method which has much to recommend it, inasmuch as the water is rendered sterile, but loses none of its salts or gases in the process. The apparatus is, however, expensive, requiring elaborate electrical apparatus and skilled attention. It is in use in some towns abroad.

Sterilisation of water may be effected by the ultra-violet rays created in Cooper-Hewitt lamps made from transparent quartz. A continuous supply of sterile water is available in five minutes. The water is unaffected as far as taste is concerned, as it retains all natural gases and salts in solution. So far the process is not widely adopted.

In Clark's process for softening hard water, Moor and Hewlett showed that bacteria were reduced, but results

were inconstant. In the Porter-Clark process, where the precipitated calcium carbonate is removed by filtration through canvas, considerable reduction in bacterial content results (Hewlett and Nankivell).

Houston's 'excess lime' method involves the addition of lime till the water is decidedly alkaline. After a period of five to twenty-four hours, the time required to kill colon bacilli, sufficient stored water is added to deal with the excess of lime. In his Tenth Research Report Houston mentions that, with small quantities of lime above the neutralisation point, colon bacilli lived over two days, whilst with 6·3 parts of lime in excess they lived for less than one day.

CHAPTER XXII

DISINFECTION AND DISINFECTANTS

Fire.

Destruction by fire is the most efficient and simplest form of disinfection, and should be employed where articles are of little value, or to remove infection from surfaces which will not be appreciably damaged thereby.

Forbush and Fernald's apparatus consists of a portable tank and pump, from which paraffin gas oil is driven through a hose (such as is used for the delivery of oil), to which is attached a pole, consisting of an iron pipe 12 feet long, which is protected by a covering of wood, and to the end of which is attached a cyclone nozzle. The fine spray from the nozzle is ignited, and the resulting torch-fire passed over the surfaces to be disinfected.

Heat.

Most articles sent for disinfection are of an organic nature, to which injury is inevitable at temperatures not much above those necessary for disinfection. Death of the bacteria is determined by the coagulation of the protein. With unlimited moisture protein free from salts can be coagulated as low as 50° C.; in the absence of moisture it can stand exposure to 170° C.

Dry Heat depends for its penetration on conduction and convection, which are slow processes, and does not kill all organisms at a temperature which can be borne by any ordinary fabric, except horsehair, even when the organisms are exposed on the surface. It is, therefore, inadmissible for disinfection of fabrics. The temperature of a dry-heat disinfector must not rise above 120° C., or woollen material will be damaged. Wide variations of temperature occur within the disinfecting chamber, owing to unequal diffusion of the gases and radiation from the heated surfaces.

Moist Heat.—Steam at any temperature and pressure which can condense without cooling is called 'saturated steam,' and will wet the surface on which it condenses. When moisture is present in a disinfector, steam condenses in the pores of fabrics, more steam is sucked in to fill the place of that condensed, and this process continues till the interior of the fabric becomes so hot that condensation ceases.

When by contact with a hotter surface, or by being derived from a saline solution, its temperature is raised above that at which it can condense under its existing pressure, the steam is called 'superheated.' The disinfectant value of strictly superheated steam is about the same as that of hot air. In practice, the extent of superheat present in a disinfector is usually not sufficient to prevent the steam from being rapidly reduced to saturation, and acting as saturated steam. It is only in the later stages of a disinfection that there is risk of the objects being too hot to cool the steam to saturation, and of organisms on the surface thus escaping disinfection. A more certain objection to the use of superheated steam is that its temperature, not being determined solely by its pressure, cannot be read off on a pressure gauge.

If the coagulation theory of heat disinfection is correct, the disinfectant effect of hot air or of superheated steam will be considerably less than that of saturated steam or hot water. The presence of air in steam has the effect of delaying or preventing the condensation of the steam, and should have a marked effect in reducing the efficiency of the steam. The temperature of saturated steam increases directly as the pressure, and on any theory, therefore,

steam under pressure must have a higher disinfectant value than steam not under pressure.

Steam is used either confined under pressure or as a current with or without a pressure exceeding that of the atmosphere. The advantage of some amount of pressure of saturated steam, however small, is that it gives a real control over the temperature of steam, which in a well-designed disinfector is practically uniform throughout. It has also been repeatedly shown that in the absence of pressure the temperature and disinfectant value of the steam depend largely on its velocity, and the rate of stoking will largely affect it—an objection which is serious because there is no convenient or trustworthy means of controlling either the velocity of the steam or the rate of stoking. What the temperature should be is still a matter of discussion. Taking all the facts together, the least exposure which can assure general disinfection is fifteen minutes to air-free, saturated steam at 115° C. This exposure may leave absolutely no margin of safety, and the conditions of saturation, freedom from air, temperature, and time of exposure must be rigidly assured independently of the operation.

Spray Disinfection.—In spraying, the operator should begin at the bottom of a wall and work upwards. If the opposite is done, the deposited liquid runs in streams down the dry wall, and staining results. Given an efficient disinfectant, a sufficiently fine spray, and a conscientious worker, spray disinfection of rooms is most satisfactory.

For descriptions of sprays and steam disinfectors, see 'Applied Bacteriology.' For action of light, see p. 9; for action of desiccation, see p. 8; for sterilisation by filtration, see pp. 215, 239.

Chemical Disinfectants.

A disinfectant, or germicide, is a substance capable of killing bacteria; an antiseptic inhibits bacterial growth; and a deodorant prevents or absorbs foul smells.

Theories of Chemical Disinfection.*—Paul and Krönig suggest that the degree of ionisation of a solution has an important bearing on its disinfectant efficiency. It is

* For further information on the theories of disinfection and on the influence of the constitution of disinfectants on germicidal power, see Professor Sommerville's 'Aids to Public Health.'

certain that in most cases the extent and rate of disinfection to be had from a solution depend on the extent and rate of its penetration into the bacterial cell, and that accordingly an increase of concentration above that of dilute solutions may not be accompanied by a proportionate increase of disinfectant power. Adsorption of the disinfectant is the first phase in disinfection, chemical action of the disinfectant on the micro-organism being the second phase. The sum, however, of all the known facts fails to establish any general chemical criterion by which the germicidal efficiency of a substance can be foretold.

Where salts of an analogous composition are concerned, disinfection is found to be more efficient as the amount of dissociation increases. Paul and Krönig found that solutions containing a toxic ion in the same proportion have an identical toxic action. Mercuric chloride and mercuric bromide are almost equally dissociated in solution, and are of equal power as disinfectants in dilutions of 1 gramme molecule in 64 litres. Mercuric cyanide undergoes less dissociation, and is a weaker disinfectant in dilutions of four times the strength. The efficiency of a solution of mercuric chloride is considerably diminished by the addition of sodium chloride, which illustrates the general principle that, when to a solution containing free ions similar ions are added, there is a tendency to re-form the original molecules.

Water ionises most electrolytes; glycerin and acetic acid are indifferent dissociants; while substances dissolved in chloroform, ether, or benzene ionise but little or not at all.

Chick found the destruction of bacteria by water between 45° and 55° to be a consistent time process, and to run parallel to the heat coagulation of proteins.

Madsen, Nyman, and Chick have shown that relatively larger number of organisms are killed when contact with disinfectant commences than after, when the rate of killing gradually falls. They found that if the results be plotted, ordinates showing the numbers of surviving organisms and abscissæ the corresponding times, the points lie on a hyperbolic curve. The curve is expressed by the formula

$$\frac{}{t_2 - t_1} \log \frac{n_1}{n_2} = K,$$

when n_1 and n_2 are the numbers of bacteria alive after the times t_1 and t_2 respectively, and K is a constant.

Delépine defines an 'antelethal inhibitory period' existing before actual death. He also shows that some disinfectants, when dilution is pressed beyond the 'ultimate lethal dilution,' actually excite growth of bacteria.

Gössl found some disinfectants to owe their efficiency towards yeast cells to the fact that they are soluble in the cell-lipoids.

Behaviour of Chemical Disinfectants.—In oil or alcohol disinfectants lose all or most of their activity. The value of alcohol itself as a disinfectant is also lessened when it holds other things in solution, but tincture of iodine is an exception. Among fats, lanolin alone seems compatible with the disinfectant efficiency of substances exhibited in it, probably because it holds them in a fine emulsion of their watery solutions (Gottstein). Some disinfectants form an emulsion on the addition of water. Rideal and Walker found that a resin soap emulsion of tricresol had three times the germicidal value of an oleate solution of the same disinfectant.

The temperature at which the organism is exposed to the disinfectant has a considerable influence on the extent or rate of disinfection. Up to the optimum temperature at which the organism to be disinfected grows on the medium in which it is exposed, the activity of a disinfectant may fall off as the temperature rises, owing to the increased vigour which the organism derives from the improvement in its conditions in respect of temperature. On the contrary, as cooling below the optimum temperature proceeds, the organism gradually passes into what Christian calls 'a state of coma,' where the bacterial cell has a correspondingly low tendency to undergo chemical change. In practice, the latter alternative seems more frequent —that is, diminution in temperature lessens disinfectant action. A relatively small difference of temperature—two or three degrees—may make an appreciable difference in the activity of disinfectants, and in their examination failure to remember this has led to serious error. Above the optimum a rise of temperature increases the activity of the disinfectant, sometimes to an enormous extent.

Fasson, Ponder, and Sims Woodhead, comparing emulsified disinfectants (cresols, etc.) with carbolic acid,

found that at lower temperatures the activity of the emulsion is raised more rapidly than that of the solution, but at the higher temperatures used the activity of the emulsion is no longer increased in proportion to the increase in the activity of the carbolic acid.

Two disinfectants used together may be more efficient than either separately. Conversely, mixture of others may diminish or neutralise the efficiency of the components. One species of bacteria may be many times more sensitive to one disinfectant than to another, when both substances exert an equal effect on a second species. In a less degree, varying degrees of susceptibility to the same disinfectant are exhibited by different strains of the same organism. Repeated subculture on favourable media will often increase resistance to chemical disinfection. By culture in media containing non-toxic proportions of a disinfectant, an enhanced degree of resistance to this disinfectant may be acquired temporarily.

Chick and Martin find that finely particulate matter affects the value of emulsified disinfectants containing the higher phenols more than it does phenol, and the loss is the greater the finer the emulsion. The removal of an emulsion of higher phenols by organisms is a process of adsorption at first, in which the organisms are surrounded by concentrated disinfectant, and so disinfectants of this class possess a higher efficiency. Kingzett and Woodcock, however, found that the fall in germicidal value generally shown when disinfectants are examined by the Lister Institute method was not due to the *mechanical action* of the solid matter (fæces). They showed that when kieselguhr, powdered pumice, and precipitated chalk were introduced into the water used to dilute the disinfectant (Sanitas Bactox), there was no depreciation in its coefficiency.

Little is known of the destruction of the filterable viruses by chemical agents. Saponine does not affect bacteria, but Cockayne (*Medical Press*, February 12, 1913) says it will kill filterable viruses, with the exception of those of trachoma and *Cyanolophia gallinarum*. These two also resist the action of taurocholate of soda, which destroys the others.

Points of Efficient Disinfectants.—1. Capability of killing bacteria. A disinfectant capable of efficient action

when diluted many times is to be preferred, not only because of the saving in storage and transit, but so that in cases of necessity, as when sporing organisms require disinfection, a stronger disinfectant dilution is permitted.

2. Retention of efficiency in the presence of such organic matter as is likely to be met with in practice. Organisms are seldom offered for disinfection separate from appreciable amounts of oxidisable organic matter, and a disinfectant dependent for its efficiency on oxidation properties wastes itself on dead matter more readily than it attacks living protoplasm.

3. Production of emulsion or solution in all proportions.

4. Permanent homogeneity. Disinfectants of a certain type separate out on standing or in cold weather into layers very different in efficiency, and although a shilling bottle may be rendered homogeneous by shaking, the matter is less easy when a 40-gallon cask is concerned.

5. Solvent power for grease.

6. Stability at all reasonable temperatures, so as to allow its use when heat is also available.

7. Freedom from toxicity in all dilutions necessary for complete disinfection.

8. Non-caustic, non-corrosive, harmless to fabric, and without action on dyes.

Acids.—All acids are disinfectants, the efficiency being directly proportional to the degree of acidity. Sulphur dioxide is obtained by burning sulphur ('Sulphur candles' are convenient), or from the liquefied gas. Plenty of moisture is necessary in the atmosphere of the room or ambulance to be disinfected, and a high temperature assists the action. Its power of penetration can be taken as practically nil, and its chief application at the present time is the destruction of vermin, notably rats, which in plague-infected ships and houses may often be desirable. For this purpose the Clayton apparatus has been used, but its high cost and somewhat complicated construction have prevented it from obtaining an extended application. Christian mentions that air containing 5 per cent. of Clayton gas kills rats, fleas, and other vermin in a few seconds, and when 1 per cent. only of the gas is present the rats cannot reach their runs. (As an alternative, Nocht and Giemsa proposed carbon monoxide for rat destruction. This gas is not a disinfectant, though

quickly poisonous to mammals and birds. As it may be inhaled without the slightest indication of its presence till the man falls senseless, its use is attended with serious risk.)

Some use has been made of crude sulphuric and hydrochloric acids in dilute solution for the disinfection of fæces, and strong sulphuric acid has been used for the disinfectant destruction of infected carcasses.

Sulphur is used with some success in destroying *Oïdium* on vines, and other fungi parasitic for plants. Marcille attributes the effect to the small amount of sulphuric acid present in the preparations employed.

Alkalies and Soaps.—Efficiency here depends on the character of the metal, but is also affected by the degree of alkalinity. Fæces may be disinfected by caustic lime, used generally as a 20 per cent. milk, but lumps must be broken up. Kaiser's method consists in adding enough hot water (50° to 65° C.) to cover the motion, and then adding about one-fourth of the entire bulk of calcium oxide, covering the receptacle, and allowing it to stand for two hours. The hydration of the lime generates sufficient heat to destroy typhoid. Lime is inefficient against the more resistant organisms, and lime-whiting is no sufficient precaution against them. Addition of a suitable disinfectant renders the process efficient. Ordinary size distemper has been shown by us to have no action on the colon bacillus. Few disinfectants are compatible with soap, binoxide of mercury and the best coal-tar products being notable exceptions. Disinfectant soap in the removal of grease, dirt, pus, etc., certainly assists in eradicating bacteria, but the period of contact of the soap in ordinary ablutions is insufficient to produce sterility. Nevertheless, as Rideal has pointed out, organisms perhaps of an infective type left on the soap by one person may remain long enough for the disinfectant incorporated to act before another uses the soap. This point is of importance in the prophylaxis of barber's rash, for which purpose disinfectant shaving-soaps are prepared. Commercial carbolic soaps are generally worthless.

Halogens.—When dry, chlorine, bromine, and iodine are poor disinfectants. Their use is practically confined to solutions, and in practice they have to be used in an

excess proportionate to the amount of organic matter present. When, as in the case of water, organic matter is practically absent, disinfection is assured by a very small percentage. 'Chloride of lime' (a mixture of calcium hypochlorite, hydrate, and chloride), hypochlorite of soda (chloros), and hermitine are the best known sources of available chlorine. The last is prepared for distribution at Poplar by the electrolysis of a solution of sodium chloride and magnesium chloride. Sodium hydroxide is added to fix free hypochlorous acid, and acts as a preservative. Much dissension has arisen over the value of this disinfectant, some authorities regarding it as stable and powerful even in the presence of sewage or other organic matter, and others as unstable and, in the presence of organic matter, untrustworthy.

The use of chlorine as a gaseous disinfectant has produced bronchitis and laryngitis, with a fatal ending. Iodine employed as a 2 per cent. solution in potassium iodide solution, or, even better, as an alcoholic tincture, is a disinfectant of the first order for the primary treatment of wounds, and for the disinfection of skin previous to operation. The tincture is recommended in the War Office memorandum. Its use in the field has extended its popularity. It is true that it stains the skin, and that serious inflammations have resulted from the injudicious use of tincture of iodine as a local application, but these are trivial details compared with its efficiency (see also p. 248). Delorme, Medical Inspector-General of the French Army, points out that dressings impregnated with perchloride of mercury and carbolic acid while iodine is being used are apt to cause a troublesome dermatitis, which does not occur when a simple aseptic dressing is resorted to. The iodine of iodoform is gradually liberated in contact with the enzymes of pus, blood, etc. Its success is largely due to this continual production of fresh iodine. Iodoform by itself is inert. Antiformin (p. 68) actually dissolves proteins, mucus, and most bacteria, and for many purposes is an excellent disinfectant. Christian mentions a 5 per cent. solution as suitable.

Metals.—Some metals, especially copper and its alloys, gradually kill adherent bacteria. In a liquid containing bacteria, a sterile zone develops round the metal. Twenty-four hour contact with bright copper plates has been

suggested in America for the destruction of typhoid bacilli and cholera vibrios in drinking-water.

When the metal is very finely subdivided, as it is in the colloidal state, its disinfectant powers increase. Colloidal silver and colloidal mercury are credited with amazing efficiency.

Inorganic Salts.—Solutions of mercury salts are strong disinfectants, efficiency depending on the proportion of mercury in dissociation. As pointed out by Sommerville and Ainslie Walker, mercuric chloride has been assigned a much greater disinfectant value than is justified, as a result of workers failing to recognise that when the medicated organisms or spores are transferred to the subculture tube a small quantity of mercuric chloride is carried over, which exerts an inhibitory action on the organisms, and the subculture tube shows no growth. But if a drop of sulphuretted hydrogen water be added, the mercury is converted into an inert sulphide, and permits the growth of organisms that otherwise would not take place within the time limits of the experiments. Mercuric chloride is poisonous, and with proteins or soaps forms compounds which have no germicidal action. The addition of hydrochloric acid (as in the L.G.B. formula) largely counteracts this depreciation. Cheatle (*Medical Annual*, 1915) appears to regard this combination with protein as a point in favour of the salt: 'Mercuric chloride is adsorbed by skin, and will remain in a soluble condition for a long time. In wounds it is adsorbed by its own precipitated albuminate, and is given up into solution slowly. Therefore it possesses a marked and most desirable depôt action.' The binoxide is strongly disinfectant in a potassium iodide solution, and is not affected by proteins to the same degree (see also p. 251). Soluble silver salts, chiefly used in ophthalmic work and for cauterising dog-bites, are powerful disinfectants, weaker than perchloride, but far less sensitive to proteins. They are incompatible with chlorides, except in certain organic combinations, from which silver chloride is only partially precipitated.

Iron and zinc salts have been credited with useful disinfectant action, but their value is of no practical account. Zinc chloride, copper sulphate, sodium fluoride, and zinc fluoride are used for preserving timber.

Copper sulphate in a proportion of 1 part per million has been used for the destruction of algæ in reservoirs. Bordeaux mixture, a popular insecticide for plants, is a mixture of calcium hydrate and copper sulphate. Springer finds that copper salts are highly selective, being most efficient in inhibiting the action of putrefactive organisms.

Oxidising Disinfectants.—The halogens (pp. 242, 251) are examples of oxidising agents. The permanganates have considerable germicidal power when in strongly acid or alkaline solution, but the readiness with which they are affected by organic substances makes them unreliable for practical use. Peroxides and ozone are open to the same objection, and have less disinfectant power.

While ozone is a deodoriser, it masks rather than destroys smells. Hill and Flack found that one part in a million irritates the respiratory tract, and even less reduces respiratory metabolism, and rapidly causes a fall of body temperature. They describe its beneficial effect, as popularly believed in, as a myth, and think the irritation of the olfactory nerves may relieve the monotony of close air. Ozone may conceal faults in ventilation when used for removing frowsiness in the atmosphere of a closed place.

Hydrogen peroxide has been used as a milk and cream preservative (p. 221). Hinks (*Analyst*, December, 1915) has shown that it persists in such material for months, and does not, as might be thought, quickly break up in the presence of organic matter. It is used for bleaching purposes, as a mouth-wash, and as lotion for infected or suppurating wounds. It is non-toxic, practically non-irritant, and does not precipitate albumen, but a free exit must exist for the escape of the oxygen, which is so rapidly liberated when the peroxide comes into contact with blood or pus. A small quantity of mineral acid or acetanilide is added to render it more stable.

Disinfectants of the Aliphatic Series.—Formaldehyde is supplied either in the form of a 40 per cent. solution (formalin) or as solid para-formaldehyde. As a gaseous disinfectant little more than superficial disinfection is possible. Disinfection is best insured by saturation of the air with moisture, maintenance of a good room-

temperature, sealing of the room, the use of at least 60 grammes of formaldehyde per 1,000 cubic feet (preferably more, up to 120 grammes), and, in the case of large rooms, mixture of the gas with the air of the room, either mechanically or by the provision of a multiplicity of inlets for the gas into the atmosphere. In the Alformant lamp twenty-five tablets of paraform are volatilised for every 100 square feet of floor space. The Kuhn lamp generates formaldehyde by the partial oxidation of methyl alcohol. In the Trenner-Lee retort and Lingner apparatus formaldehyde gas and water vapour are passed into the room. The 'Maine process' requires only a 10-quart tin pail, in which 300 grammes of potassium permanganate are placed and 600 c.c. of formalin are added. The reaction is violent, and is complete in about five minutes, a maximum dosage of the room being quickly obtained. Base modifies the method by using 300 c.c. of water with 375 grammes of potassium permanganate and 600 c.c. of formalin, but finds the yield of gas less satisfactory. The Military Commission in Vienna, on the contrary, got better results by dilution of the strong formaldehyde solution. Where portability of materials is important, paraform and sodium bicarbonate solution may be substituted for formalin.

On evaporation, molecules of formaldehyde condense to form para-formaldehyde (polymerisation), $(HCOH)n$, which has little disinfecting power, but which, on heating, produces formaldehyde. Polymerisation seems to be prevented to a large extent by the presence of moisture (see also above). As a spray, formalin can be used in any ordinary apparatus. It is stated by McLaughlin that if the formaldehyde be mixed with phenol vapour, polymerisation does not take place, and that therefore the formaldehyde penetrates thoroughly. The mixture he employs is 3 parts of 40 per cent. solution of formaldehyde with 1 part of carbolic acid. It may be volatilised in a retort, or it may be simply poured on a sheet, which is then hung up in the room to be disinfected. Base quotes the conclusions of Werner as to the practical requirements for an effective formaldehyde disinfection: (1) 5 grammes of formaldehyde (absolute) should be present in each cubic metre of space (0·1416 gramme per cubic foot), and should be allowed to act for seven hours;

(2) the temperature of the room should not fall below 50° F., the best temperature being from 68° to 77° F.; and (3) the strength of the formalin should be ascertained.

Ethyl alcohol in from 20 to 50 per cent. solution is germicidal for many vegetative forms of bacteria. Stronger mixtures generally have less effect *in vitro*, but, all the same, rectified spirit is said to be admirable for the disinfection of the surgeon's hands. If bacteria thereon are not quickly killed, it is argued that the alcohol fixes them on the skin so effectively that they can only be removed with difficulty (see also p. 248).

Picric acid is used for external disinfection in 0·5 per cent. aqueous solution, especially after burns or scalds. For wounds, Philip Turner recommends a 1 per cent. solution in methylated spirit. Chloroform is used for media (p. 39). Quite recently urea in strong solutions has been recommended for antisepsis of wounds.

Disinfectants of the Aromatic Series.—Phenol (carbolic acid) in saturated solution contains 6 to 7 per cent. Proteins and organic matter generally only slightly lessen its value. It is very poisonous and caustic, prolonged application to a surface producing 'carbolic acid gangrene.' It is useless for sporing organisms, and is a comparatively weak disinfectant. Its value in the arts is such that very little of it is left in commercial carbolic acid, which consists mainly of the cresols and higher phenols. Cresols are only slightly soluble in water. A mixture of ortho-, para-, and meta-cresols (tricresol) is stronger and more soluble than individual cresols. Cresols may also be dissolved in alkaline solution.

Cresols are much reduced in efficiency by proteins. In saturated salt solution the disinfectant value of crude carbolic acid is greatly increased. With crude sulphuric acid it forms, if the mixture is conducted under conditions preventing any great rise of temperature, a substance miscible with water, and possessing strong disinfectant activity. For disinfection and destruction of fæces this sulphonated cresol may be used in 5 per cent. solution, not, of course, in metal utensils.

Ordinarily neutral tar-oils with no appreciable disinfectant value are left in or mixed with tar distillate, and the saponified product produces an emulsion with water. Innumerable products of this type are made. Their

efficiency varies not only with their active ingredients, but also with the character of the emulsion which they form, from about the same as that of phenol to about three times as much. Commercially they are known as soluble carbolic acid, soluble creosote, etc. Creolin is a type of numerous preparations of the same character. They are all poisonous and sensitive to proteins. If naphthalene is present in excess, it is deposited on standing in cold weather. Lysol contains 50 per cent. of the cresols in fat or linseed oil saponified with addition of alcohol. It gives a clear solution with water, having slightly less efficiency on naked bacteria than cresol, much superior solvency for grease, and equal sensitiveness to proteins. Many preparations of the same type are now sold, and are convenient for such laboratory purposes as disinfecting slides and glass apparatus after use.

Liquor cresolis saponatus (British Pharmacopœia, 1914) consists of cresylic acid 50 per cent. dissolved in castor oil and saponified with potassium hydrate.

A new era in chemical disinfection began when Ainslie Walker first prepared cyllin, which contains oxidised hydrocarbons possessing a diphenyl nucleus, emulsified with a neutral hydrocarbon oil. A large number of emulsified disinfectants can now be obtained—kerol, M.O.H. fluid, bactox, and cofectant being the most reliable.

It should be noticed that the use of water containing very high total solids (*e.g.*, sea-water) reduces the efficiency of these preparations, and special articles, in which glue is used as a base, are made for such waters; but all such preparations require to be agitated before dilution, owing to the fact that they separate out on standing into layers of very different efficiency.

Salicylic acid was formerly used as a preservative for milk and milk products. It is still used for preserving jams and lime-juice. It has recently been used as a dressing for wounds. Thymol is a strong disinfectant that enters into the composition of many mouth-washes and tooth-pastes.

Disinfectant Powders.

If a suitable powder be sprinkled on manure-heaps, dustbins, or in pail-closets or privies, not only are foul odours absorbed, but flies are kept away, and prevented

from laying eggs or feeding on the refuse. Lime, which has a high capacity for absorbing sulphuretted hydrogen, is very well adapted as a base for a disinfectant powder. This is inadmissible with phenol, but is successfully employed in the preparation of cyllin and kerol powders.

Examination of Disinfectant Powders.—Robertson and Severn's process (Rept. M.O.H. Cape Colony for 1906): One hundred grammes of the powder are thrown into a litre stoppered measure, and made up to the mark with sterile distilled water (temperature to be between 15° C. and 18° C., little or no difference being noticeable between these limits). The cylinder is shaken violently and horizontally every fifteen minutes for four hours, and left then for subsidence. At the expiration of twenty-four hours, some of the clear supernatant extract is drawn off with a sterile 50 c.c. pipette into a sterile bottle or flask. This is used to make the dilutions. (In some cases a layer of the finer particles of the base will remain obstinately on the surface of the extract, and some will stick to the pipette. Quickly wipe this away with a sterile cotton-wool plug.) Each 10 c.c. of the extract is regarded as equal to 1 gramme of the powder. As a standard, a 15 per cent. carbolic powder is taken as 1. Such a powder is difficult to prepare and keep and extract, owing to its deliquescence and the necessity for using hot water for its complete extraction. A 10 per cent. extract of a completely extracted 15 per cent. carbolic acid powder can be simulated by taking 100 c.c. of a 4·7 per cent. solution of phenol and diluting to 313 c.c. (or 100 c.c. 5 per cent. phenol made up to 332 c.c.). This solution is called "standard powder 1 in 10." Then a 17 dilution, for instance, is made by taking 100 c.c. and diluting to 170 c.c. The R.-W. process is then applied to extract and standard.

Bacteriological Examination of Disinfectants.

Few disinfectants are capable of being examined by chemical means, so that the disinfectant value is revealed, and, after all, the determination of the germicidal value by actual experiment on living organisms is the only reliable method of gauging the value. Many factors, however, enter into the question, which must be recognised: the time allowed for action, the temperature of

medication, the age of the culture, the species, and even the strain, of the organism, the number of organisms presented for disinfection, the reaction of the culture medium, and the influence of organic matter, must all be considered. The desirability of stating the values of disinfectants in terms of a common standard is obvious, as it allows comparison, and the standard invariably used is absolute phenol (first suggested by C. G. Moor). The examination of a disinfectant involves, first, the determination of its value in comparison with that of phenol when the organisms are presented 'naked'—*i.e.*, without any appreciable amount of extraneous organic matter present; and, secondly, when such organic matter as is likely to be met with in practice is introduced into the test to ascertain what, if any, is the depreciation in value caused thereby. One method only of testing a disinfectant against a naked organism (the Rideal-Walker method) need be considered in any detail, as this particular method has been universally adopted for the purpose. It requires a certain amount of dexterity and much care to perform, but it is remarkable for the manner in which factors likely to cause discrepancies are recognised and guarded against.

Rideal-Walker Method.—A special test-tube rack is used: a lower tier contains five holes for four tubes, holding the strengths of the disinfectant to be tested, and one tube containing the standard phenol. The upper tier has spaces for thirty test-tubes, in six sets of five holes. In the upper tiers are placed tubes of standard broth, and the tubes are numbered 1 to 30.

The method may now be briefly described as follows: To 3 c.c. of a particular dilution of the disinfectant in sterile water add 3 drops of a twenty-four hours' blood-heat culture of the organism in broth; shake, and take subcultures every two and a half minutes up to fifteen minutes. Incubate these subcultures for at least forty-eight hours at 37° C. Allowing thirty seconds for each act of medication and the same time for making each subculture, four different dilutions of the disinfectant under examination, together with one standard control, may be tested against the same culture, under conditions which make the results strictly comparable. No table is to be considered complete which does not show a positive result

in the first column and a negative result in the last. The strength or efficiency of the disinfectant is expressed in multiples of carbolic acid performing the same work—*i.e.*, when a dilution of the disinfectant has been obtained which does the same work as the standard carbolic acid dilution, the former is divided by the latter, and so a ratio is obtained which the authors call the 'carbolic acid coefficient.'

The use of the term 'carbolic acid coefficient' in connection with later tests, giving different results, has led to much confusion. To guard against this, it is advisable to express the results as 'Rideal-Walker' coefficients when employing the original test.

The following table shows the degree of refinement to which this test may be carried with a little care:

B. Typhosus (Kral), Twenty-four Hours' Broth Culture at 37° C.

Room-Temperature, 15° to 18° C.

Sample.	Dilution.	Time Culture exposed to Action of Disinfectant (Minutes).						Subcultures.	
		2½	5	7½	10	12½	15	Period of Incubation.	Temperature.
Disinfectant W	1 : 70	×	×	...	...	...	...	48 hours	37° C.
,,	1 : 80	×	×	×	...	...	...	,,	,,
,,	1 : 90	×	×	×	×	×	...	,,	,,
,,	1 : 100	×	×	×	×	×	×	,,	,,
Carbolic acid...	1 : 80	×	×	...	...	...	.	,,	,

From this it is seen that a 1 in 70 solution of disinfectant W has the same action as a 1 in 80 solution of carbolic acid. Disinfectant W is therefore not quite so active as carbolic acid, and this is represented by the carbolic acid coefficient—viz., $\frac{70}{80} = 0{\cdot}87$.

Notes on the Test.—For rough work it may be taken that 110 parts by weight of acidum carbolicum liquefactum (B.P.) contains 100 parts by weight of absolute phenol. Carbolic acid sometimes contains alcohol, which

has been said to increase its germicidal power, and therefore to give a low coefficient for the sample. But, in fact, the amounts found are too small to have appreciable influence. More important is the quantity of cresols usually found in carbolic acid crystals. As cresol has approximately three times the germicidal power of phenol, the error from this may be considerable. The solidifying-point of the crystals should be not less than 40° C. Ainslie Walker and Weiss (*Journ. Franklin Inst.*, 1912, 101) say the bromine titration method does not yield trustworthy results as regards the purity of the phenol, but it may be employed for checking the strength of the 5 per cent. stock solution prepared from pure phenol. The 5 per cent. stock solution should be kept in a closely-stoppered bottle in a dark cupboard. The culture must be a Lemco broth one, twenty-four hours old. The broth should be made as follows:

Lemco	..	..	20 grammes.
Peptone	..	..	20 „
Sodium chloride	..	..	10 „
Water	..	..	1 litre.

Boil the mixture for thirty minutes, and standardise to a reaction of +1·5 per cent. (+15, Eyre's scale), using phenolphthalein.

When the cholera vibrio or diphtheria bacillus is the test organism, a neutral or alkaline broth must be used ('Bacteriological Examination of Disinfectants,' 1907, p. 25), and for the latter a forty-eight hour old culture is necessary (*ibid.*, 17). Hewlett and Norman Hall (*Journ. Hygiene*, 1912, p. 473) have shown that when working with anthrax spores agar is preferable to nutrient broth for the subcultures, as the latter does not show a life very often when agar does.

Before addition to the medication tubes the culture should be freed from clumps. This may be attained by shaking the culture and allowing to settle for twenty minutes before the test, when the culture required is pipetted from the top half, or it may be filtered through filter-paper.

The general laboratory procedure is to keep a stock culture on agar, and subculture on to a fresh agar slope at the end of a month From the twenty-four hour

broth culture a fresh subculture is made in broth before the former is used for the test. This subculturing into fresh broth may and should be done every twenty-four hours, whether a test is to be made or not. After a month a fresh agar slope is made, and from the month-old agar streak a fresh series of broth cultures started.

All measures, pipettes, and tubes should be sterile, and sterile distilled water must be used for making the dilutions.

The pipette used for delivering the three drops of culture to the disinfectant dilutions should deliver 0·1 c.c. per drop. The needle used should be composed of thin aluminium rod, with a short piece of thin platinum wire passed through and twisted round an eye in the end of the rod. The free end of the wire is then made into a loop about 4 millimetres in diameter. If the sides of the loop be depressed so that in side-view it looks like a cholera vibrio, the loop easily carries a big drop. Use of a *thin* wire allows quick cooling.

When subculturing, a good-sized drop (easily obtained by bringing the loop away from the liquid with a jerk) should always be taken up.

The desired result in the phenol column is life in two and a half minutes and in five minutes, and no life thereafter. Rideal and Ainslie Walker (*Medical Press*, September 15, 1915) suggest that a typhoid culture be rejected if it calls for a phenol dilution higher than 1 in 110, or lower than 1 in 90.

While the test is in progress, the temperature of the medication tubes should not vary more than 2° C. When tests are being performed on successive days, it is of considerable help if the temperature of the room in which the first test is done be known. Should the temperature be higher the next time, a weaker phenol solution should be used, or, if lower, a stronger one.

Ainslie Walker (*N.Y. Med. Journ.*, February 1, 1913) points out that the use of bullock's heart broth instead of Liebig broth has the effect of depressing a coefficient by about 50 per cent. For further details of this test, see 'The Bacteriological Examination of Disinfectants,' by W. Partridge.

A coefficient obtained on a freshly prepared emulsion is termed a 'temporary coefficient,' but as frequently a

disinfectant solution is made up and allowed to stand for several days before it is completely used, so if the dilution necessary is calculated upon a freshly made emulsion, which afterwards separates, and which in this way has a lower efficiency than that calculated, it is quite conceivable that considerable damage might be done. Rideal and Walker, therefore, suggest that before applying the test to a disinfectant a 1 per cent. solution should be prepared at normal temperature in a stoppered litre flask, the portion required for further dilutions in the test to be pipetted *from the top* of the flask after twenty-four hours' subsidence, avoiding the withdrawal of any insoluble matter which may be floating on the surface. The coefficient is then referred to as a 'permanent coefficient.'

The *Lancet* Commission (see *Lancet*, November 13, 20, and 27, and December 18, 1909; *Pharm. Journ.*, July 30, 1910) used a considerably modified Rideal-Walker test. *B. coli* was used as the test organism, MacConkey's bile-salt broth for the secondary subcultures, and platinum cups were substituted for the inoculating needle. The test covers thirty minutes as against the fifteen minutes for the Rideal-Walker test, and more dilutions both of the disinfectant and phenol are used. In determining the coefficient, the weakest dilutions of disinfectant and phenol killing in two and a half minutes are compared, and a coefficient for the thirty-minutes period is obtained in the same way. The mean of these two figures is taken. This method is no advance on the Rideal-Walker. When bacteria have been exposed to non-lethal dilutions of disinfectant, they are 'sick.' To put these into MacConkey media, which cuts out weak organisms, introduces an indecisive factor into the comparison. Anderson and McClintic, of the United States Public Health Service, advocate keeping the typhoid bacillus as the test organism, as there is greater variability between strains of colon bacilli than between typhoid strains.

Anderson and McClintic (*Journ. Infect. Dis.*, viii. 1), in their 'Hygienic Laboratory Method,' compare an average of two and a half and fifteen minute periods, and use an increased number of dilutions of both disinfectant and phenol. So much work has to be done in the time that

seeding tubes have to be left unplugged. The process is no improvement on the Rideal-Walker test.

Organic Matter in Disinfectant Testing.—Many forms of organic matter have been suggested for incorporation into the bacteriological test. Milk of various strengths, washings from schoolroom floors, and fæces, have been suggested and used; but these either (in the case of milk) never want disinfection in practice, or allow no comparison between two disinfectants, owing to the impossibility of different workers using identical material.

Sommerville and Walker are undoubtedly right in insisting that the forms of organic matter included in the test should be of a simple form. The false coefficients obtained with oxidising disinfectants can be revealed in a most satisfactory manner by the use of urine, mucin, peptone, casein, gelatin, or blood. Sommerville and Walker first dilute the disinfectant in the proportion recommended by the manufacturers or sanctioned by use, all further dilutions being made with the organic solution. The disinfectant is allowed to remain in contact with the organic solution for one hour before adding the test organism. They prefer as a diluent a solution containing 0·5 per cent. gelatin and 0·5 per cent. rice starch, the latter being added to meet the needs of adsorption. A coefficient obtained with this diluent and allowing the preliminary hour's contact of organic matter with the disinfectant is known as a 'Sommerville-Walker coefficient.'

In the 'Lister Institute method,' the fæces are dried, first in a water-bath and subsequently at 105° C., ground to a fine powder in an agate mortar, and passed through a fine sieve with a mesh of 130 to the inch. Quantities of 0·15 gramme are added to test-tubes, to which 2·5 c.c. of distilled water are added, and the tubes are sterilised. Different amounts of a suitable dilution of the disinfectant are added to each tube, together with enough distilled water to make 5 c.c. This gives different concentrations of the disinfectant in presence of 3 per cent. of fæces. The tubes are inoculated in the same way as when the test is made with distilled water. Fifteen minutes is allowed for the disinfectant to act; the test is done at 20° C., and an exactly similar experiment is done with phenol.

A disinfectant must be applied in such a strength as to leave an excess above the proportion theoretically needed. In other words, what Defries has termed 'a wide margin of safety' must be insisted on. Sommerville and Walker suggest 'it might be insisted that the multiple 5 be applied as a minimum to the strength of the various disinfectants which are found to perform the same work as 1 in 100 phenol.'

APPENDIX

Insolubility of Enzymes.—Enzymes exist in a colloidal state, and are insoluble in the true sense of the word. Bayliss (*Jour. Physiol.*, December, 1915) shows urease, lipase, emulsin, invertase, lactase, papain, peroxidase, and catalase are active in media from which they can be filtered off by ordinary filter-paper, leaving an inactive substrate.

Streptococci in Fæces.—Distaso (*Lancet*, January 8, 1916) points out that though Wright's 'saddle-bag' streptococcus (see p. 135) is a common organism in fæces, it is not *S. fæcalis*.

Tubercle Bacilli.—Eastwood and Griffith (*Jour. Hygiene*, January, 1916) found 21·1 per cent. of human bone and joint tuberculosis cases to be due to bovine bacilli. The bovine type was also identified in three out of seventeen cases of tuberculosis of the human genito-urinary tract. They emphasise the need for caution when, as commonly happens, a 'human' strain not in full vigour gives a poor growth on glycerinated test media. Should such a strain cause fairly severe lung disease in a rabbit, there is a risk of it being wrongly returned as 'bovine.'

Meningococci.—Nankivell (*Jour. Roy. San. Inst.*, January, 1916) is convinced that cerebro-spinal fever is not propagated by personal contact, and believes it to be conveyed by the bite of some animal parasite; all his cases had shown flea, bug, or louse marks. Symonds and others do not think vermin necessary for the spread

of the disease. Nankivell also thinks the period of incubation to be short—not more than four days, probably twenty-four to forty-eight hours. Meningococci having been found in the blood in pure culture when meninges and cord were unaffected, Nankivell strongly recommends blood-culture.

Researches on the meningococcus till the end of 1915 are summarised in a 'Report of the Special Advisory Committee upon Bacteriological Studies of Cerebro-Spinal Fever during the Epidemic of 1915' produced by the Medical Research Committee (National Health Insurance) and to be obtained from Wyman's, Fetter Lane, E.C. (6d.). In this report, Professors Andrewes, Bulloch, and Hewlett express scepticism regarding the 'pleomorphism' of the meningococcus. The absence of growth at 23° C., formerly thought to be of diagnostic value, has proved unreliable; some meningococci show a degree of growth at this temperature, though it is never profuse. Further, certain forms of *Micrococcus flavus* refuse to grow at 23° C., and Gaskell finds that, at this temperature, *Micrococcus catarrhalis* gives only a feeble growth that soon fails. Nasgar and *war-nasgar* have proved unsatisfactory for throat swabs. Agar or nasgar, smeared with a few drops of fresh human blood are used by some workers in preference to other media, but pea-flour-trypsin-agar (in its final form of *Trypagar*) is also strongly recommended. The Report considers Shearer's work on the stimulus of nasal mucus on the meningococcus and Gordon's work on its inhibition by salivary streptococci.

Details of Douglas's trypsin-broth, used in the preparation of trypagar, will be found in the *Lancet*, October 10th, 1914. For subsequent work the reader is referred to numbers of *The Journal of the Royal Army Medical Corps.*

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